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TOBACCO BLUE MOULD *(Peronospora tabacina* Adam*)* IN NORTH QUEENSLAND. 2. EPIDEMIOLOGICAL STUDIES BEARING ON THE DEVELOPMENT AND CONTROL OF THE DISEASE

By W. PONT, B.Sc.,* and I. K. HUGHES, B.Sc.†

SUMMARY

In North Queensland, blue mould (*Peronospora tabacina* Adam) persists on out-of-season hosts comprising plant crops, standover crops, volunteer plants and the native host *Nicotiana glauca* Graham. There is some evidence of seedborne infection. The oospore stage of the fungus appears to be absent.

A uniform distribution of inoculum does not exist early in the season. Early mould outbreaks are predictable following a study of the location of sources of infection. The build-up of mould in early-planted crops ensures a high intensity of inoculum by the time the major field plantings are made.

Initial aerial infections are slight and may be expected three weeks after field plantings where a source of inoculum is close. Economic loss and coincidental build-up of mould to an intensity where widespread aerial dispersion of spores takes place may be expected some three weeks after initial infections. The relationship of rate of spread to intensity of inoculum is discussed.

Stem mould is correlated with leaf mould severity at an early age and is found to increase as time from leaf infection increases.

A north-south row direction was found to alleviate mould losses, probably because of better penetration of sunlight.

The relationship between rainfall and the development of the disease is discussed. Dew alone is sufficient to ensure the spread of blue mould throughout a crop but the increase in intensity of infection to epidemic proportions depends on adequate rainfall.

I. INTRODUCTION

In accordance with a resolution passed at the fourth meeting of the Queensland Tobacco Advisory Committee, a comprehensive survey of the incidence of blue mould (*Peronospora tabacina* Adam) in tobacco crops in North Queensland was carried out by the junior author in the 1958 and 1959 seasons. Much of the information presented in this article has been assembled from observations made in the course of this survey. In addition, use has been made of experimental data and observational evidence accumulated by the senior author during work on various aspects of the blue mould problem at the Parada Tobacco Experiment Station from the 1956 season onwards.

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Tobacco in North Queensland is now grown mainly as an irrigated crop. The acreage grown under irrigation is increasing yearly and it is expected that upwards of 12,000 acres of irrigated tobacco will eventually be planted each season in the Mareeba-Dimbulah district alone.

Irrigation has enabled planting to be carried out much earlier in the year than was the case when the crop was rain-grown and transplanting to the field necessarily coincided with early summer storm rains. In the main tobacco districts of the north the majority of first field plantings are made in August. This provides a continuity of plantings extending from August through till early October or even later in the year if the few remaining rain-grown crops are included. In some years in some districts, particularly those along the Burdekin River, off-season crops which are planted in February-March and harvested during the winter are common. Of recent years, the time of planting of the earliest plantings of the main crop has been progressively moved forward until in 1960 the first crops were transplanted in April-May. If this planting policy is persevered with, it will mean that there will be tobacco in the field for most of the year in these districts.

Seedbed blue mould has waned in importance in North Queensland since the benzol vapour fumigation treatment was adopted by growers and remains a problem only when benzol treatment is neglected or carelessly handled.

Substantial losses are, however, caused by blue mould in field plantings. The effect of field blue mould on the tobacco plant has been described previously (Pont 1956, 1959). In estimates made by Angell (1957) the percentage loss in crops due to the combined effects of leaf mould and stem mould ranged up to 44 per cent. The surveys carried out by Hughes in 1958 and 1959 showed that losses due to leaf mould were as high as 40 per cent. He assessed the average loss per farm surveyed from leaf and stem mould at 11·4 per cent. in 1958 and 13·6 per cent. in 1959. In neither year did late rains with attendant epiphytotics develop and in neither year could mould be regarded as severe.

II. METHODS

(a) Data from Field Surveys, 1958 and 1959

The field surveys conducted in the 1958 and 1959 seasons involved all the major tobacco-growing districts and were carried out from mid-May to December in each year. In 1958, 173 farms and in 1959, 183 farms were surveyed. Each of these farms was visited at intervals of three weeks. On each farm a datum area of approximately 2 ac of tobacco was selected and this was taken from the first field plantings.

(i) 1958 Survey

The information sought and disease assessments made at the respective inspections were as follows for the 1958 survey.

(1) *Seedbed Phase.*—Seedbed notes comprised:—plant variety; benzol usage (in both volume and evaporating surface used to a unit area of seedbed); frequency of fumigation; time of commencement and cessation of fumigation; type and condition of seedbed covers (an airtight cover is the aim of North Queensland tobacco growers, the covering material being galvanized iron or plastic sheeting); preplanting seedbed sterilization; watering practices; proximity of inoculum where obvious; condition of seedlings; date of field planting; and presence or absence of sporing blue mould. Seedbeds were re-inspected and examined for sporing blue mould at the date of field planting.

(2) *Field Phase.*—Four field inspections were made of each datum area.

First Inspection.—Three weeks after field planting the percentage of mould-affected plants for each datum area was established. Eight random lots each of 50 consecutive plants were examined for the presence of sporing mould. The percentage of plants affected was calculated from these 400 plants.

Notes were taken on fertilizer application; irrigation and cultivation; use of fungicides; plant height, colour and texture; and, where of interest, farm topography and soil type.

Second Inspection.—Six weeks after field planting mould severity was assessed as follows:

$$\text{Severity} = \frac{\text{Number of plants infected}}{\text{Number of plants inspected}} \times \text{Average severity rating for 100 plants inspected.}$$

The number of plants inspected was the same as at the first inspection—eight random lots each of 50 consecutive plants and in addition four lots each of 25 consecutive plants on which individual severity ratings were made; in all, 500 plants.

All severity ratings were made on the leaf showing most infection. The rating used was:

0—Nil infection.

1—Scattered spotting.

2—Concentrated spotting.

3—Spotting involving more than 50 per cent. of the leaf area or one leaf lost per plant.

Again at this inspection the numbers of irrigations and cultivations were noted, as well as primings, use of fungicides, plant height, colour and texture and approximate date of harvest.

Third Inspection.—Nine weeks after field planting, percentage mould loss figures were compiled for each datum area. Mould loss (calculated as percentage number of leaves lost) was assessed for each plant in eight random lots of 25 consecutive plants. The average percentage loss for each datum area was calculated as the average of these individual ratings. In addition, the number of plants obviously affected with stem mould or systemic mould was recorded by noting the number of plants lodged, stunted or deformed in eight random samples of 50 consecutive plants. Irrigation, cultivation, fungicide usage, crop height, colour and texture were noted as before.

Fourth Inspection.—This inspection was conducted after harvest had commenced (that is, 12-15 weeks after field planting). To estimate systemic mould incidence, stems were cut on one side near ground level and the presence or absence of brown discolouration recorded. Eight lots of 25 consecutive plants were cut in each datum area. When complicating factors such as leaf-miner tunnelling contributed to a stem discolouration no results were recorded.

At this inspection notes were made on row direction and row and plant spacing.

(ii) 1959 Survey

For the 1959 season figures on average price per pound of leaf on sale and returns per acre were kindly supplied by the Tobacco Leaf Marketing Board. These are figures for the whole of the growers' leaf, which was made up in part only from the datum area.

A random selection of farms was made in the 1958 survey, but in 1959 blocks of farms were selected and considerable time was taken to locate and record preplanting sources of inoculum in order to study the spread of blue mould. As districts were studied as a whole in this year, individual plantings of different age were being inspected. The second field inspection was modified by omitting the severity rating. No stem cutting was carried out in 1959.

(b) Data from Experimental Plots, Parada

In the experimental work at Parada Tobacco Experiment Station, records of epidemiological observations were made by charting the block in question and recording at weekly intervals the position of all fresh blue mould infections. Infection dates were regarded for the purposes of this work as the dates on which infection was visible.

The figures representing intensities of infection are values for average percentage blue mould infection per leaf calculated for the datum plants in the randomized unsprayed plots in blue mould control experiments. In arriving at these average values each leaf was assigned a figure for percentage blue mould infection based on a visual estimate of the percentage of the lamina showing

blue mould lesions. The expression total amount of blue mould/number of leaves then gave the average value in percentage per leaf. In order to obtain a truer estimate of the amount of damage, the figure employed for "number of leaves" was the average number of live leaves in the best spray treatment plots, and where the number of live leaves in the plot under consideration was less than this the leaves lost were recorded as being 100 per cent. mould-affected.

Stem mould infections were recorded by raising the bark at the base of a plant at ground level on both sides of the stalk in a line parallel to the row and recording the presence or absence of the characteristic discolouration. By sampling various portions of the block for stem mould incidence at successive dates and by referring to time of infection as shown on the block maps, it was possible to correlate stem mould incidence with date of infection.

III. SOURCES OF INOCULUM

(a) Standover and Volunteer Plants

In most of the North Queensland tobacco-growing districts a succession of tobacco plants exists from season to season. These plants comprise undestroyed crop residues, stem and root suckers from these, and seedlings which emerge following seed dissemination by wind or mechanical means. The perpetuation of the fungus on such material provides inoculum for seedbeds and field plantings, a fact which has been described fully by Hill and Angell (1933). The absence of standover and volunteer plants in some districts is reflected in the absence of mould in seedbeds and in the delay in its appearance in field crops.

Farm inspections in the Burdekin district prior to seedbed plantings showed sporing blue mould on volunteer plants on almost all farms. This district grows alternate crops which are irrigated and seedling growth within these crops is abundant. In the Dimbulah district volunteer plants showed on very few farms, resulting in an average distance from infection of over one mile at the time of preparation of seedbeds. In the Mary River district no infection could be found on volunteer and standover plants at the time of seedbed preparation; this district is situated over 20 miles from the nearest tobacco-growing area.

The spread of the pathogen from the stem of a tobacco plant into new axillary shoots has been described in southern Australia (Hill and Angell 1933; Mandryk 1960). Although in North Queensland axillary and root suckers commonly show blue mould infection, it is doubted that this infection arises from internal stem infections. In an isolated district of North Queensland, standover plants were inspected for blue mould infection. Although these plants had experienced severe mould in the previous season, and at the time of inspection axillary suckers were plentiful, no blue mould was recorded. These plants would have endured summer temperatures above 104° F maximum, which may have been responsible for the non-survival of the fungus (Lucas 1958).

(b) Nicotiana glauca

Nicotiana glauca has been known to be a host of *P. tabacina* for many years. In North Queensland it appears to be confined to the Burdekin River catchment area and is ecologically confined to the immediate area of the river bank. Preferring broken soil, it grows frequently on eroded embankments. Plant inspections in 1959 showed very few plants infected and all of these were on the eastern river bank and were subjected to morning shade. Stem infection was observed in plants severely affected. The short-term eradication of this host would be practical.

(c) Oospores

In the United States of America Wolf (1939) collected oospores in necrotic leaves during each of seven seasons and stated that they may be presumed to occur wherever the pathogen exists. According to Wolf, McLean, and Dixon (1936) and McGrath and Miller (1958), primary blue mould infection of seedlings in North America is usually caused by the thick-walled overwintering oospores. These spores are not common in southern Australia (Adam 1925; Angell and Hill 1932). In North Queensland, microscopic examination on numerous occasions of necrotic leaf tissue and infected stems has failed to locate oospores.

A study of cropping histories on different lands and the time of appearance of blue mould indicates that the oospore stage of the fungus is of no importance in North Queensland.

(d) Seedborne Infection

Evidence of seed transmission of blue mould was supplied by Angell (1929) and Angell and Hill (1932).

A study of blue mould outbreaks in well isolated districts where the possibility of aerial infection is unlikely indicates that seedborne infection may be one source of blue mould in North Queensland.

Some such isolated outbreaks may be related to the use of particular seed stocks. One seedbed planting with over 20 miles isolation and no local source of inoculum comprised 10 seedbeds. Two-and-a-half of these were sown to one unsterilized seed stock while $7\frac{1}{2}$ were sown on the same day to another stock. Infection occurred in the first $2\frac{1}{2}$ seedbeds, while none occurred in the remaining beds. The farm had not grown tobacco or seedlings before and all seedbeds were similarly treated and planted to the same variety.

The importance of seedborne inoculum is difficult to assess in the field. Many growers begin benzol fumigation soon after seedling emergence and any infection which might show is corrected and passes unobserved.

During the 1959 season 50 per cent. of North Queensland's tobacco was grown from seed saved by individual growers and their friends. Much of this seed was not treated with silver nitrate.

(e) Seedbeds

Since the discovery of the efficacy of benzol as a seedbed fumigant, seedbed mould has become less of a problem. Correct seedbed management using the recommended schedule of 1 sq. in. of evaporating surface to 1 sq. ft. of airtight seedbed cover applied every third night has in all cases achieved effective control of mould. There is therefore now no essential reason for seedbed mould to be a source of field infection.

In the 1958 season a survey of benzol fumigation on 138 farms showed that 28 per cent. of the farmers were using ineffective schedules. However, only 9 per cent. of the growers experienced seedbed blue mould. In the 1959 season, on 131 farms inspected 30 per cent. of the seedbeds were being ineffectively fumigated with benzol; only 13 per cent. of them experienced seedbed blue mould. A study of the sources of inoculum and the isolation distances involved shows that the discrepancy between the number of farms prone to seedbed infection and the number actually infected is due to escape from infection.

(f) Masked Infection of Seedlings

Angell (1957) stressed the importance of what he termed "masked infection" in seedlings when, due to the use of inadequate benzol and dry conditions, blue mould can infect the seedlings but the usual symptoms, observable on plants in the open, are masked. Following transplanting blue mould immediately appears on the developing leaves of infected seedlings.

Mandryk (1957) investigated the effect of different concentrations of benzol vapour applied under glass-covered iron trays at various intervals after inoculation with *Peronospora tabacina*. Among other things he found that higher concentrations of benzol (20 ml and 30 ml per 115,943 c.c.) applied in later stages of incubation (3-4 days after inoculation) usually did not eliminate the pathogen in all plants and resulted in masking of the disease.

The standard Departmental recommendation for North Queensland of ½ pt (284 ml) of benzol apportioned between two containers each 5 in. x 4 in. for each 10 ft x 4 ft x 1 ft iron seedbed cover gives a concentration approximately equivalent to that obtained by Mandryk with his 30 ml dosage.

It has been consistently demonstrated in Departmental experiments and by successful growers that this dosage, when properly applied every third night, prevents the development of sporing blue mould in seedbeds. Due to this fact and to the phytotoxic effects of benzol when applied under vapour-tight covers

even when treatment every third night is practised, more frequent treatment has not been considered desirable. During the 1958-1959 surveys, field inspections three weeks after planting, when presumably any masked infection would have shown up, disclosed no evidence of this occurring.

In order to investigate the possibility of masked infection following the standard seedbed recommendations, a series of three seedbed experiments, in which the frequency of benzol application was varied from the normal, was carried out at Parada Tobacco Experiment Station during the 1957-1959 seasons.

In the three trials the treatments were applied to small beds 4 ft x 2 ft under iron covers 4 ft x 2 ft x 1 ft; 80 ml benzol was exposed under each cover in an evaporating tray 4 in. x 2 in. x 1 in. supported above the seedlings in a central position. Two treatments were employed in each experiment. These were replicated five times in a randomized block layout. Covers were applied before 4.30 p.m. and removed at 7.30 a.m. and benzol residues were measured each morning. About two weeks before transplanting and on a day following a night on which both treatments coincided, the seedlings were watered with a heavy suspension of conidia of *P. tabacina*.

At transplanting time seedlings were pulled at random from each plot. In the field the treatments were further randomized and three field plots, one in each of three blocks, were planted from each seedbed plot to give a 10 x 3 layout.

Results were assessed by weekly counts of mould-infected plants and by examining each plant for stem mould infection 9-11 weeks after planting out and prior to commencement of harvest.

The following treatments were applied:—1957: benzol 1 sq. in./1 sq. ft. every night and every third night; 1958 and 1959: benzol 1 sq. in./1 sq. ft. every second and every third night.

Experiment 1 (1957) was abandoned because of the pronounced phytotoxicity of the nightly benzol treatments. Many of the seedlings receiving this treatment were killed. Growth of the remainder was retarded so much that none was suitable for planting out. Seedlings treated every second night were in both 1958 and 1959 less thrifty (more stunted and lighter in colour) than those receiving treatment every third night. The size difference persisted for the first 3 or 4 weeks in the field but by harvest time no appreciable differences could be noted.

Results of the 1958 and 1959 experiments are presented in Table 1. There is no indication that masked infection had occurred in the beds receiving less frequent treatment.

Table 1

Leaf Mould and Stem Mould Development in Plants Which had Received Benzol Treatment Every Second and Every Third Night

Year	Treatment	After 5 Weeks		Percentage Infected Plants After 8 Weeks	Percentage Stem Mould Prior to Harvest
		Total No. of Plants	No. of Infected Plants		
1958	2nd night	354	110	88	8
	3rd night	359	76	90	5
1959	2nd night	395	1	100	50
	3rd night	404	1	100	44

In 1958 minimum temperatures ranged from 43°F to 62°F during the course of the experiment. Volume concentrations (calculated on the basis of a 10 ft x 4 ft bed) ranged from 165 to 330 ml, with a mean for the whole experiment of 235 ml. In 1959 the minimum temperature range was 31°F to 64°F, and volume concentration 175 to 320 ml with a mean of 220 ml.

IV. DISTRIBUTION OF INOCULUM

(a) Means of Distribution

The shedding of conidia from the conidiophores can apparently be brought about either by a reduction in relative humidity or by mechanical shock. Pinckard (1942) found that conidia were produced but not released in a saturated atmosphere. Mature spores were shed when the atmosphere subsequently became drier. Cruickshank (1958) concluded that either change in humidity or mechanical shock may be involved in conidial discharge, that each may act independently of the other and that their effect increases with the magnitude of the change in relative humidity or the size of the shock. Waggoner and Taylor (1958) investigated the dispersal of spores from blue mould lesions and found that evidently the maximum number of spores are dispersed early in the morning and few or no spores are airborne during the afternoon or night. They estimated that a blue mould lesion produced about 2 x 10^5 spores per day; however, only 1 spore in 4 x 10^3 produced a lesion even at close range. Despite this dilution factor and the apparent very limited viability of the conidia in the absence of water and the presence of sunlight (Lucas 1958; McGrath and Miller 1958), the disease is wind-borne for considerable distances in North America. For example, Stover and Koch (1951) attributed widespread outbreaks of the disease in Ontario, Canada, to wind-borne conidia from Kentucky or Ohio, over 125 miles to the south-west.

In addition to airborne spread, other means of transport have to be considered. For instance, the moths of the tobacco leaf miner, *Gnorimoschema operculella* Zell., have been demonstrated to be vectors of the disease (Angell, Hill, and Currie 1930). It seems possible, therefore, that other insects may carry the conidia on their bodies.

In the course of the surveys in North Queensland in 1958 and 1959, instances of what appeared to be infection due to the carriage of spores on the person were noted. In such cases the first blue mould spots in a tobacco field appeared near points of entry to the planting. Undoubtedly the movement of persons from one crop to another and one district to another aids the distribution of blue mould inoculum.

The distribution of infected seedlings is a very important means of mechanical spread of blue mould. In this way inoculum may be transported many miles in a very short time. Mould-affected seedlings are generally easily available for distribution among growers who are short of planting material, whereas a reliable source of mould-free seedlings is usually more difficult to obtain.

The inevitable result of planting infected seedlings is severe economic loss from stem mould and such plantings are frequently abandoned at an early age. A far more widespread and more important aspect is the establishment early in the season of a concentrated source of inoculum.

Angell (1957) drew attention to the relation of blue mould infected seedbeds to losses from field mould on farms in the Mareeba-Dimbulah district. He quoted a mean percentage crop loss of 6.86 on farms where healthy seedlings were used, compared with 25·3 per cent. in crops which had been planted with diseased seedlings.

(b) Characteristics of Airborne Spread

Although mechanical spread may be important in establishing new infections early in the season, airborne dispersal of inoculum is by far the most important means of inoculum distribution.

Survey work has shown the presence of inoculum prior to planting at an average distance of one mile from most seedbed or field sites, but by no means must a uniform distribution of inoculum be presumed to exist. Early in the season, outbreaks of blue mould most often occur close to sources of inoculum and early outbreaks are predictable following a study of the location of these sources.

When plantings are made from infected seedbeds or when later in the season groups of farms become infected, the inoculum concentration is increased many thousands of times and a general and uniform airborne distribution of spores may occur. In the 1959 season late in September such a fall-out of spores occurred and enveloped all farms to the west of Mareeba, some of these 14 miles from the source of inoculum, which was in this case 100 or more infected farms in the Mareeba district itself (Figures 2–4).

Spore survival of the fungus is considered a very important factor in airborne infection. Survey work showed that when dispersion of spores takes place at a time when conditions favour spore viability, all farms exposed to infection become infected. The amount of infection is roughly proportional to the area of leaf exposed, indicating that cultural practices and varietal characteristics lend little or nothing towards plant resistance.

There is the suggestion that a continual fall-out of viable spores does not exist; rather a fall-out of viable spores produces infection and then no further infection takes place until a spread of blue mould occurs from the first points of infection.

As initial infections by air are usually slight, it is uncommon for mould to appear before the three weeks' stage when healthy planting material is used.

(c) Spread Within a Field

Dispersal of airborne spores under conditions favouring spore viability results in a scattered field infection. The initial spots of blue mould are few and frequently overlooked by a grower.

The appearance of 100 mould spots per acre as a result of a fall-out of spores during weather conditions conducive to spore survival is high and indicates that a source of inoculum is close. A more common distribution would be 15 spots per acre.

After initial infection has taken place, the rate of spread of the disease throughout a crop depends largely on weather conditions. A normal increase of mould would result in the loss of only one or two of the lower leaves three weeks after the recording of the first mould spots in the field. After this time the build-up is likely to be much more rapid. Where infection occurs late in the life of a crop and shaded conditions prevail within that crop, severe economic loss has been noted in two weeks.

Mechanical transference of inoculum, and airborne spread providing isolated foci of infection, have provided opportunities to study the airborne spread of blue mould from point sources of infection. The rate of spread from such sources has been recorded in three instances. Successive spore generations could be observed as the fungus spread in roughly circular stages away from the source. The damage at the source of infection was in each case much more than at the perimeter and the result a "hole in the crop." The rate of spread of the fungus is sufficiently slow for this effect to be conspicuous.

In all three cases, within the first three weeks mould spots appeared only as far as 300 yd from the focus of infection. In the following week a spread of one mile was recorded in the one district where contiguous farms enabled

observations to be made. This indicates that the concentration of inoculum at a source must be considered to fully understand the field rate of spread of blue mould within a district.

In Figure 1 is shown the rate of increase of blue mould in one field sampled in 1959. On each of 17 days from September 3 to 25 at least 400 plants were examined in random counts of 50 plants and the presence of blue mould noted. No leaves were removed and first infections were noted soon after the plants were three weeks in the ground.

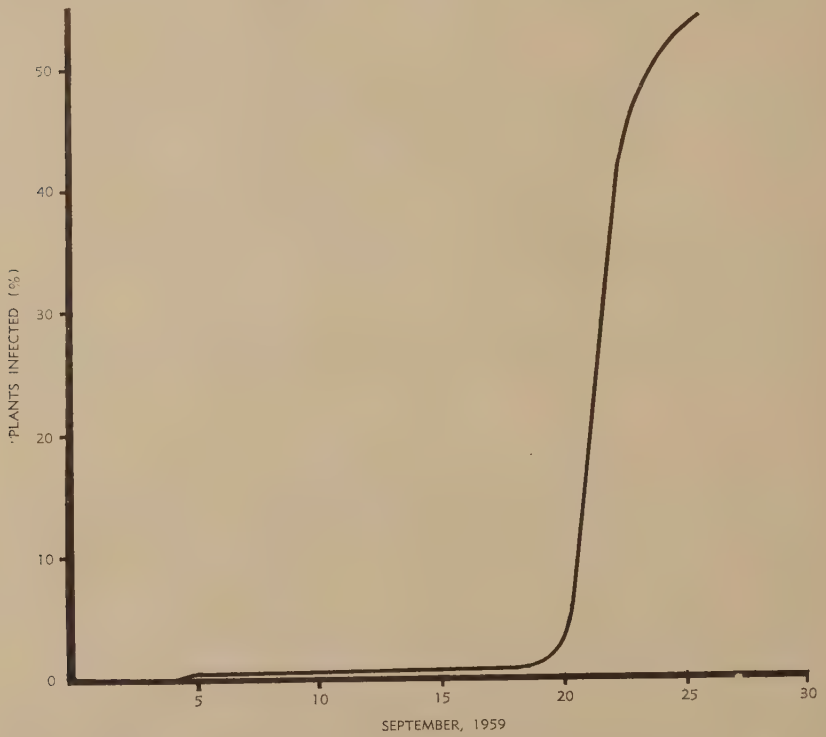

Fig. 1.—Illustration of the rapid build-up of blue mould three weeks after first appearance. Field in Mareeba district sampled September, 1960.

From this graph may be seen the low initial infection leading to a sudden field build-up in three weeks.

This rate of increase is considered representative of most North Queensland farms. The weather conditions corresponding to the above period were cool and cloudy in early September with rain recorded on September 1, 2, 5, 10 and 12. Total precipitation was 0·17 in.

(d) Rate of Spread within a District

As mentioned earlier, local outbreaks of blue mould early in the season occur close to sources of infection. When discussing the rate of build-up on an individual farm it was noted that district rate of spread is dependent to some extent on the concentration of inoculum at a source. It is not unusual to find a build-up of mould taking place on a single farm or on a number of farms without spread from these areas. Spore survival is considered the most important factor in rate of spread of blue mould throughout a district. As the distance of spore travel will affect spore viability, distances between farms within a district and between districts will determine the district rate of spread.

The series of maps in Figures 2-5 shows the progress of the disease throughout the Mareeba-Dimbulah districts in 1959. Although a few minor sources of infection such as groups of volunteer plants occurred, the uniformity of distribution of infection within fields throughout the Dimbulah district leaves no doubt that the source was the heavily infected Mareeba district.

Figure 2 shows the geographical distribution of blue mould in field plantings on August 10. This early mould was due to transplanting mould-affected seedlings.

Figure 3* shows the distribution on September 10. A local spread of blue mould has occurred embracing farms up to four miles distant from the original source of infection. When compared with Figure 4 it can be seen that outlying districts escaped early infection due to their isolation. However, once a large source of inoculum was built up at Mareeba, aerial infection served to distribute inoculum to all outlying districts.

Figure 4 shows the geographical distribution of blue mould in field plantings on October 10. By the end of October mould had increased in farms in the outlying districts to cause economic losses.

Figure 5 shows the geographical distribution of lodging from stem mould. Data for this map were collected in late November. From comparison with Figure 3 it is noted that stem mould lodging has occurred only where plantings experienced early and severe mould due to close proximity to initial sources of infection.

Many tobacco seedbeds and some field plantings escape blue mould infection. It has been determined that an isolation distance of one mile from a small source of inoculum (e.g. a group of volunteer plants) gives a splendid chance of seedbed escape from infection.

Numerous growers far removed from a source of infection do not use benzol fumigation and seldom experience blue mould attack.

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* A mould outbreak occurred at Dimbulah in a field planting made from infected seedbeds. A local spread was not recorded from this planting as all farms in the area west of Mareeba experienced a fall-out of spores from the Mareeba district.

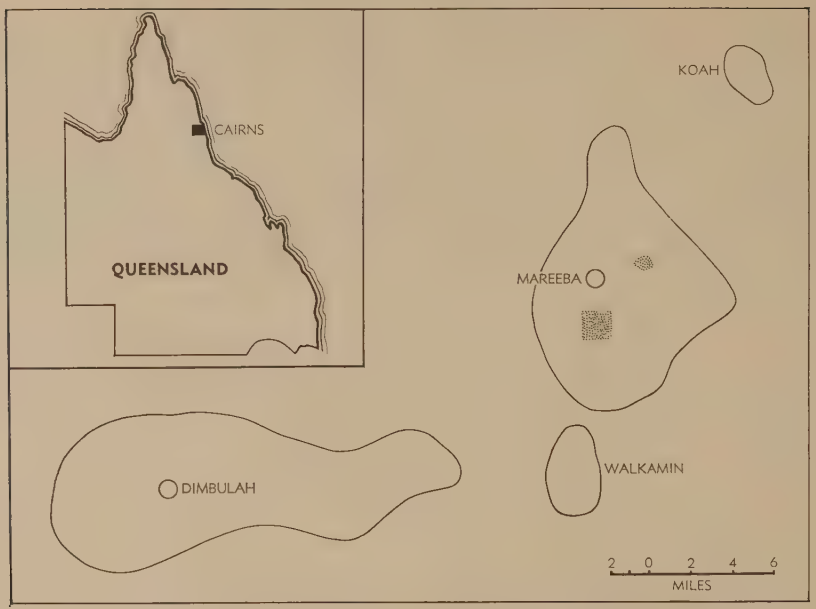

Fig. 2.—Airborne distribution of blue mould, Mareeba-Dimbulah district, 1959. Distribution on August 10.

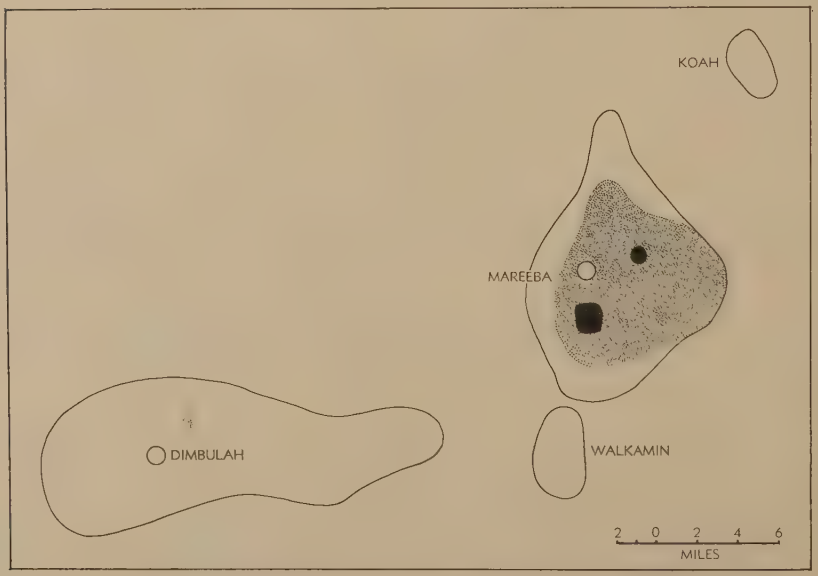

Fig 3.—Airborne distribution of blue mould, Mareeba-Dimbulah district, 1959. Distribution on September 10.

Fig. 4.—Airborne distribution of blue mould, Mareeba-Dimbulah district, 1959. Distribution on October 10.

Fig. 5.—Airborne distribution of blue mould, Mareeba–Dimbulah district, 1959. Distribution of lodging from stem mould in late November.

Escape from infection due to isolation becomes increasingly obvious after field plantings have been made. While inoculum concentrations are low, those farms closest to sources of inoculum are first infected, and those some miles removed remain healthy.

Although the evidence suggests that a large number of farms could dispense with benzol fumigation of seedbeds and rely on escape from infection, one would be reluctant to advocate such a practice, as small sources of inoculum in the form of volunteer plants are often present on a farm although undetected by the grower.

V. RELATIONSHIP BETWEEN STEM MOULD AND LEAF MOULD

During the 1958 season, 30,000 stems were cut on 150 farms and records made of the presence or absence of the characteristic brown discolouration. No stem discolouration was recorded where crops had experienced no leaf mould. The number of plants showing stem mould in a planting was found to be correlated with the severity of mould in the crop at an earlier age. In Figure 6 is shown the correlation between mould severity at six weeks of age and the percentage of plants exhibiting stem infection later. All ratings were made on the leaf worst affected and according to the method described on page 3.

In Table 2 the amount of stem mould is related to time of first infection The number of farms on which observations are based is given in brackets. As mould severity at six weeks is dependent on time of infection, the data in the table are in conformity with Figure 6.

Table 2

RELATION OF STEM MOULD DAMAGE TO EARLY AND LATE INFECTION

—	Infection before Three Weeks	Infection after Three Weeks
Percentage internal stem discolouration, 1958 ..	23·5 (49)	9·4 (81)
Percentage stem lodging, 1959	15·1 (21)	0·9 (72)

The number of farms on which the observations are based is shown in brackets.

It is apparent that almost a direct relationship exists between the severity of leaf mould at an early age and the amount of stem mould at maturity. The severity of mould at an early age depends largely on the distance of the crop from a source of infection. The avoidance of leaf mould attack at an early stage of plant development by eliminating nearby sources of infection is recommended as a practical field control for stem mould.

The relation of stem mould to date of infection is brought out in data taken from field mapping at the Parada Tobacco Experiment Station during the 1958 and 1959 seasons.

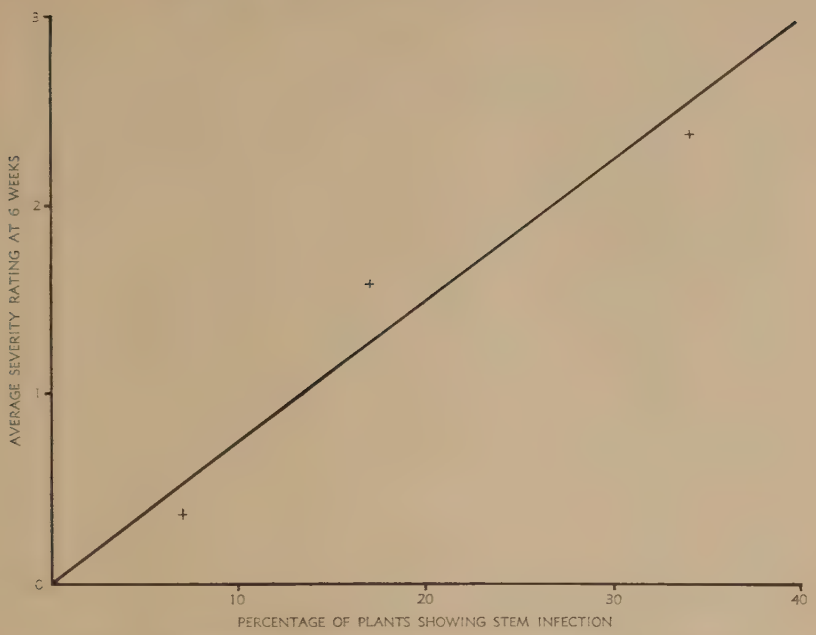

Fig. 6.—Correlation of stem infection with leaf mould severity at six weeks.

In 1958 an analysis was made of stem mould incidence in a block of over 3,500 plants, planted on September 11. The incidence of leaf mould was mapped in this block at weekly intervals from October 6, when the first diseased plants were counted, until October 27, when all plants were infected.

Stem mould incidence was determined in a series of six examinations commencing on November 14. At each inspection date the plants in portion of the block were rated as positive or negative. By December 22 all plants had been examined. The results are detailed in Table 3.

Similar data are presented in Table 4 for a block of about 1,850 plants surveyed in 1959. In this case the date of planting was September 17. Two inspections for stem mould were made, one half of the block being rated on each of the dates of November 21 and December 2.

Allowing for some uneven distribution of stem mould throughout the datum blocks, there was a tendency in both years for percentage stem mould to increase as the interval between date of infection and date of inspection lengthened. From Table 3 it will be noted that 43 days after initial field infection stem cutting revealed only 12·6 per cent. stem mould infection. It is considered from general observations that stem mould will be of little significance until a minimum of five weeks has elapsed since leaf mould first appeared.

Table 3

OBSERVATIONS ON THE RELATIONSHIP BETWEEN PERCENTAGE STEM MOULD AND TIME OF SAMPLING, PARADA, 1958

Date of Inspection for Stem Mould	14–xi–58		18–xi–58		28–xi–58		5–xii–58		12–xii–58		22–xii–58	
Date of Infection	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)
6–x–58 (26 days from planting)	36	8·3	95	12·6	96	22·9	82	25·6	35	37·1	109	28·4
13–x–58 (33 days from planting)	170	11·7	288	11·1	278	19·7	322	20·4	149	42·9	273	42·4
20–x–58 (40 days from planting)	104	10·5	172	14·2	198	28·7	161	27·9	96	54·1	174	42·5
27–x–58 (47 days from planting)	51	15·6	145	8·9	135	28·8	153	25·4	72	38·7	127	32·2
Average	..	11·6	..	11·6	..	24·5	..	23·8	..	44·6	..	38·4

Table 4

Observations on the Relationship between Percentage Stem Mould and Time of Sampling, Parada, 1959

Date of Inspection for Stem Mould	21-xi-59		2-xii-59	
Date of Infection	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)
26-x-59 (39 days after planting)	93	31	103	29
2-xi-59 (46 days after planting)	555	28	423	46
9-xi-59 (53 days after planting)	360	41	314	55
Average	..	32·9	..	47·4

There was little obvious correlation between date of infection and final percentage stem mould in these observations. This is to be expected in the light of the low intensity of infection characteristic of early infections.

Since the incidence of stem mould is proportional to intensity of leaf infection, it is to be expected that the factors which influence intensity of leaf infection affect stem mould development also. In the surveys during the 1958 and 1959 seasons this relationship was verified for row direction.

VI. CLIMATIC FACTORS AFFECTING THE INCIDENCE OF BLUE MOULD IN THE FIELD

In the United States of America the optimum temperature for the production of conidia of *P. tabacina* is evidently around 60° F. Clayton and Gaines (1933) determined the optimum at 60-62° F. They later found (Clayton and Gaines 1945) that alternating high and low temperatures (77° diurnal and 60° nocturnal) were more favourable for sporulation than a constant temperature of 60° F. However, the effect of temperature on sporulation was modified by changes in the physiology of the host because exposure to prolonged periods of low light intensity enabled sporulation to take place at temperatures above 80° F. Dixon, McLean, and Wolf (1936) considered the optimum for sporulation was somewhat lower, namely 56° F. Wolf (1957) stated that germination does not take place except in a film of water or in a saturated atmosphere. Dew provides sufficient moisture. In America, periods of cloudy, rainy weather are regarded as blue mould weather and temperatures above 85° F are definitely inhibitory to the fungus. Outbreaks of the disease are arrested by the advent of several bright days with temperatures in excess of 85° F (Wolf 1957).

In Australia, Cruickshank (1958) has investigated the critical relationship between sporulation intensity and diffusion pressure deficit of the host tissue and relative humidity of the air in the immediate vicinity of the abaxial leaf surface. Threshold values of D.P.D. and R.H. for maximum sporulation were 3–4 atmospheres and 97 per cent., respectively. The minimum period of exposure to optimal conditions necessary to give rise to maximum sporulation was found to be three hours.

In North Queensland, blue mould build-up takes place in the field in the absence of rainfall. During September and October long dry periods characterized by hot sunny days and cool nights are common. Maximum temperatures are likely to be well in excess of 85° F, but minimum temperatures seldom are higher than 65° F. Heavy dews are common. The rapid development of field mould under such conditions is illustrated in Figure 7, which shows the progressive increase in percentage infected plants in a block of irrigated tobacco at Parada Tobacco Experiment Station during October 1958. The seedlings were transplanted on September 10 and the first infections were mapped on October 6; the block was 100 per cent. infected on October 27. Also on the graph are maximum daily temperatures and mean nocturnal temperatures (2-hourly readings from 10 p.m. to 6 a.m.). The duration of any dews which occurred during this period was noted with a 7-day dew recorder (Theis and Calpouzos 1957). Only 0·02 in. of rain fell during the 7 weeks' period to October 27.

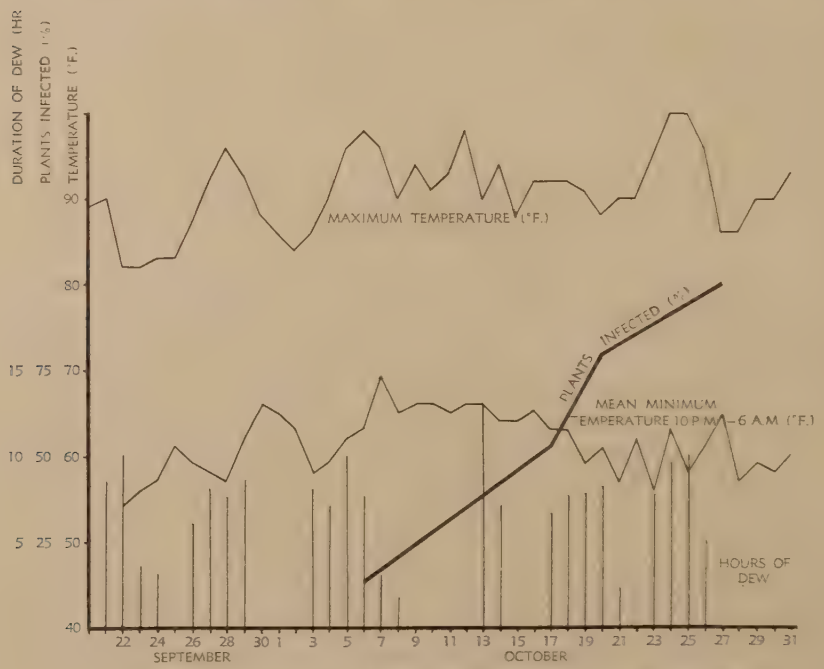

Fig. 7.—Blue mould development during the absence of rain, Parada, 1958.

Epiphytotics of the disease are, however, dependent on rainfall for their development. It is unusual if rains do not occur at some time during the growing period of the North Queensland crop and any precipitation of sufficient magnitude to be accompanied by high humidities, depressed daytime temperatures and reduced light intensity can give rise to a sudden increase in intensity of infection. Isolated storms may lead to a local intensification of the disease but a succession of storms within the one district or several days of showery weather within one week are more important contributing factors.

Experience at Parada Tobacco Experiment Station has shown that the amount of damage in fields exposed to "blue mould" weather is, nevertheless, variable and depends to a large extent on the amount of early infection. This is due to the operation of the acquired resistance effect (Pont 1959).

The main weather influences prevailing during a period which proved ideal for blue mould development in an out-of-season crop are illustrated in Figure 8. The crop in question was a mixed block of blue mould susceptible and resistant

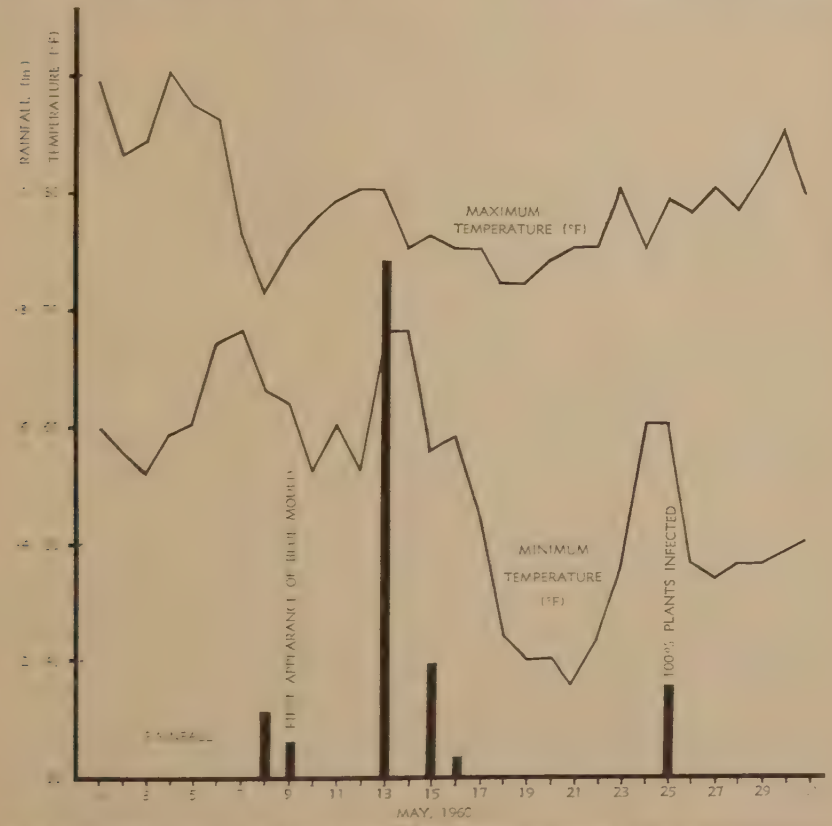

Fig. 8.—Weather conditions and blue mould development following a light initial infection in an out-of-season crop, Parada, May 1960.

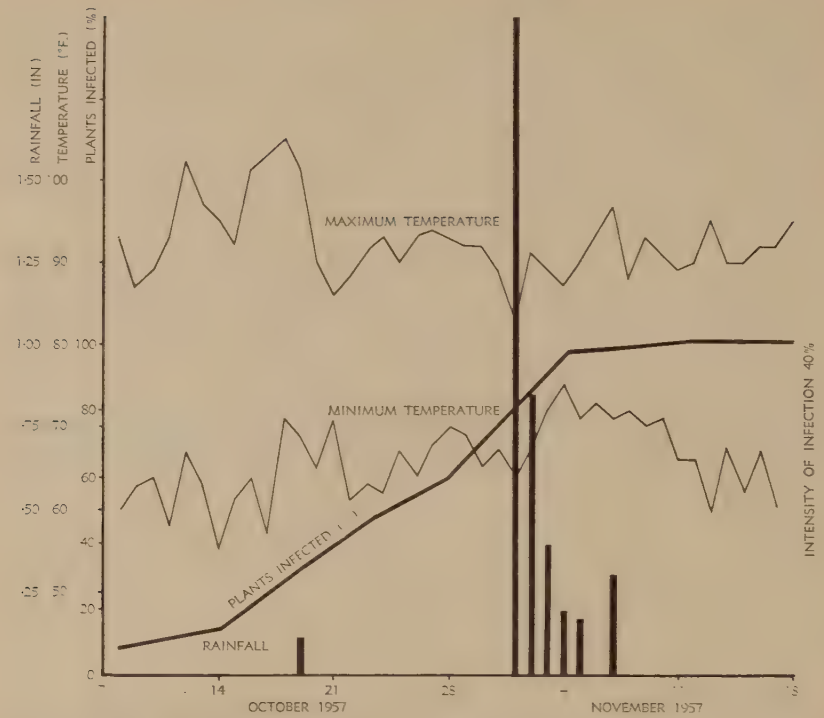

Fig. 9.—Weather conditions associated with an epiphytotic of blue mould during the normal growing season, Parada, 1957. Intensity of infection on November 18 was 40 per cent. (Compare Fig. 10.)

lines planted at Parada Tobacco Experiment Station on March 16, 1960. Blue mould was absent until May 9, when flowering had commenced, but on this date a scattered infection resulting from limited airborne inoculum was noted along the windward side of the block. Following 1·10 in. of rain during the period May 13–18, the infection spread rapidly, and by May 25 all susceptible plants were infected, with an intensity of infection ranging as high as 50 per cent.

The weather conditions associated with an epiphytotic during the normal growing season are given in Figure 9. Here the incidence of the disease in the unsprayed plots in a blue mould spray trial at Parada Tobacco Experiment Station in the 1957 season is depicted. As a comparison, the data associated with a non-epiphytotic year at the same station are presented in Figure 10. The weather data plotted are daily maximum and minimum temperatures and rainfall.

In 1957 the planting date was September 10. The first blue mould was noted on October 7. All plants were infected by November 11. The intensity of infection on November 18 was 40 per cent. It should be pointed out here

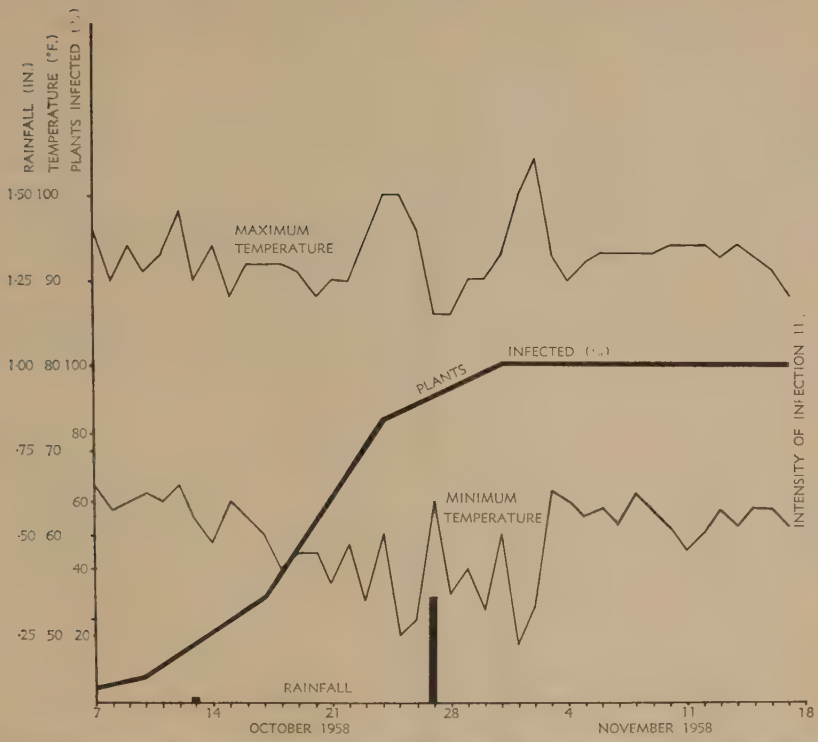

Fig. 10.—Weather conditions associated with blue mould development in a non-epiphytotic season, Parada, 1958. Intensity of infection on November 18 was 11 per cent. (Compare Fig. 9.)

that, due to their acquired resistance, plants which were infected early escaped serious damage in the November epiphytotic. The mean figure for intensity of infection has, therefore, been calculated from late infections only.

In 1958 the block was planted on September 11. Blue mould appeared by October 3 and all the unsprayed plants were diseased on October 31. The intensity of infection on November 17 was 11 per cent.

It can be seen that although the rate of spread from plant to plant is much the same in each year the intensity of infection is much greater in the year of high rainfall.

In Figure 11 weather conditions during the growing seasons of 1956 and 1958 in the Burdekin district of North Queensland are compared. The 1956 season was a wet one in this area with well distributed rainfall throughout the entire growing season of the main crop. Losses from leaf mould were heavy and the lodging of stalks weakened by systemic infection reached serious proportions.

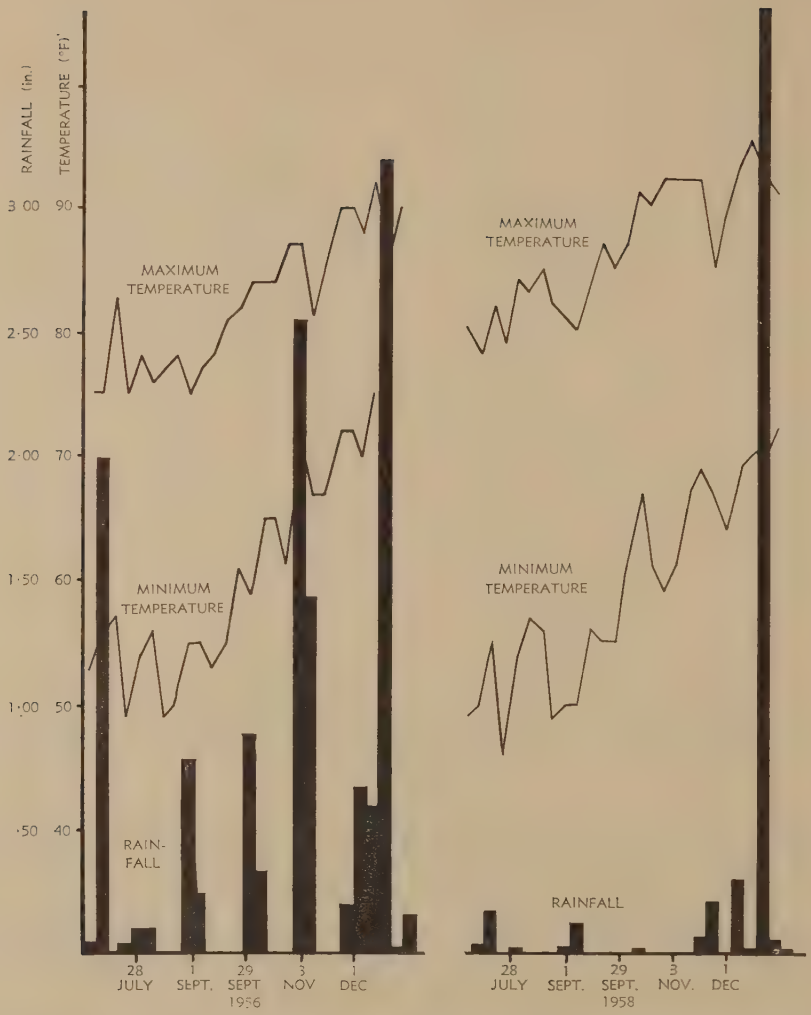

Fig. 11.—Comparison of weather conditions in a year of heavy (1956) and light (1958) losses from blue mould in the Burdekin district.

In 1958 dry weather prevailed during the growing season of the main crop. Blue mould was widespread but never severe. Systemic mould was responsible for little obvious loss.

VII. FACTORS OTHER THAN CLIMATE INFLUENCING INTENSITY OF INFECTION

It has been noted following a general airborne spore distribution such as illustrated in Figures 2-5 that the amount of initial infection per field is dependent on the leaf area exposed. That some of these plantings were young, some old, some watered and some dry, each on land with different cropping histories and frequently different fertilizers, demonstrates that plant predisposition for infection is little influenced by cultural practices.

However, once initial infection is established the intensity of subsequent infection can be influenced by several factors which will now be considered.

(a) Row Direction

The effect of row direction is very pronounced. An east-west planting favours the disease, probably due to the shading of one plant by its neighbour. The effect of morning shade was noted also when *Nicotiana glauca* plants were being inspected for blue mould infection. The plants on the eastern river bank showed infection, while those on the western bank showed no infection.

Table 5 shows an analysis of percentage leaf loss for each row direction. Recordings from different districts are listed separately, as are the years when observations were made.

Table 5

EFFECT OF ROW DIRECTION ON DAMAGE BY LEAF MOULD

	East–West			North–South		
	No. of Farms	Average Loss (%)	Range of Loss (%)	No. of Farms	Average Loss (%)	Range of Loss (%)
1958						
Mareeba–Dimbulah ..	37	10·5	0–30·6	30	7·6	0–19·4
Burdekin	26	13·0	1·7–39·6	3	7·2	6·7– 7·4
Total	63	11·7	0–39·6	33	7·4	0–19·4
1959						
Mareeba–Dimbulah ..	20	13·25	0–40	27	8·9	1–21
Burdekin	28	12·5	0–30	6	11·6	5–28
Total	48	12·8	0–40	33	9·4	0–28

An analysis of field notes reveals that there is also a difference in stem mould due to row direction. This is related to the difference in leaf mould severity at an earlier stage of growth.

During the 1958 season, recording of discolouration in cut stems was taken as an indication of the presence of stem mould. The results in relation to row direction are shown in Table 6.

Table 6

CORRELATION OF STEM INFECTION WITH ROW DIRECTION, 1958 SEASON

Row Direction	No. of Farms	Average Percentage of Plants with Stem Infection	Range (%)
East–West ..	56	19·9	0–83·5
North–South ..	36	12·8	0–68

(b) Row Width and Plant Spacing

A survey of 150 farms in 1958 indicated that leaf mould was less where plant spacings and row widths were greatest. These results were not supported in the 1959 survey. Row spacing varied from 3 ft 6 in. to 4 ft 6 in. and plant spacing from 18 in. to 27 in.

(c) Water Usage

The application of irrigation water early in the life of the crop was found in the 1958 and 1959 seasons to have little effect on leaf mould severity. Table 7 shows an analysis of percentage leaf loss at harvest time for plantings subjected to various water applications during the first six weeks after transplanting. The number of farms inspected for each group is included in brackets.

Table 7

AVERAGE PERCENTAGE LEAF LOSS FOR DIFFERENT FREQUENCIES OF WATER APPLICATION DURING THE FIRST SIX WEEKS

Year	Planting Water Only	Planting Water plus Water after 4 weeks	Water from Planting Onwards
1958	10·7 (37)	10·7 (63)	7·6 (13)
1959	10·6 (72)	9·0 (70)	7·6 (15)
Average	10·6 (109)	9·8 (133)	7·6 (28)

The number of farms inspected for each group is given in brackets.

The effect of early irrigation practice on the subsequent expression of stem mould was also investigated. Table 8 shows the percentage of plants affected with stem mould for the three irrigation groupings. The data for 1958 were obtained by stem cutting, while those for 1959 represent lodging. There is a tendency for a reduction in stem mould damage with increase in water.

Table 8

AVERAGE PERCENTAGE STEM MOULD AND LODGING IN RELATION TO FREQUENCY OF IRRIGATION

Year	Planting Water Only	Planting Water plus Water after 4 weeks	Water from Planting Onwards
1958	16·3 (66)	15·6 (59)	10·6 (15)
1959	8·0 (38)	3·8 (63)	0·1 (14)

The number of farms inspected for each group is given in brackets.

In 1958 a replicated irrigation experiment was carried out at the Parada Tobacco Experiment Station comparing normal watering, light watering and withholding water for the first six weeks. The results were statistically inconclusive.

To sum up, there is as yet no definite evidence that withholding water alleviates the leaf mould position. The average time taken from transplanting until harvesting begins is 10 weeks. This time varies with individual farmers and may be reduced to eight weeks or extended to 14 weeks, depending on watering procedure and cultural methods. It is considered that the extension of the growing period by withholding water contributes to stem mould losses.

(d) Fungicides

During the 1959 season close attention was paid to farms which used fungicides on a field scale. Zineb was the fungicide most widely used. Where growers sprayed before the appearance of blue mould, i.e. before the initial infections took place, a practical field control was obtained. In Table 9 is a comparison of zineb sprayed with unsprayed showing percentage leaf loss at harvest. All of these farms were located on the Burdekin irrigation area.

Table 9

PERCENTAGE LEAF LOSS AT HARVEST OF SPRAYED AND UNSPRAYED CROPS, BURDEKIN, 1959

Percentage Leaf Loss	0	1	2	3	4	5	6	7	10	11	12	15	16	17	19	20	26	27	29	30
Unsprayed ..	1	..	1	1	..	6	..	4	3	1	1	3	3	2	1	1	1	1	1	1
Sprayed	24	1	1	2	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..

Figures represent number of farms in each category.

The average leaf loss of 32 farms unsprayed was 12 per cent. and that of 29 farms sprayed was 0·7 per cent.

An analysis of spray concentrations and frequency of application shows that for those farms using zineb an average of 1·2 lb. of zineb in 36 gal of water was applied per acre every 4–5 days.

In the Mareeba-Dimbulah district only a few growers used zineb on a field scale. Seven growers who applied zineb had an average leaf loss of 4 per cent., while 52 who did not spray suffered an average loss of 9·8 per cent.

The success of zineb is attributed to the preventive effect of the spray. Earlier in this paper it was shown that initial blue mould infections occur through the establishment of a few isolated spots within a field and subsequent field build-up takes place from these points. Zineb spraying prior to infection seems quite capable of controlling the disease with this low concentration of inoculum.

Analyses of mould loss, zineb usage and leaf values have been compiled for the 1959 season for the Mareeba and Burdekin districts. From Table 10 it may be seen that zineb spraying does not reduce the value of the leaf. Zineb treated leaf is apparently acceptable to the manufacturers and presumably poses no taint problem. It is of interest to note also from this table that leaf value is altered very little by mould damage.

Table 10

ANALYSIS OF MOULD LOSS AND LEAF VALUES WITH AND WITHOUT ZINEB SPRAY, 1959

No. of Farms	Average Leaf Loss (%)	Zineb Usage	Average Price of cured leaf (pence per lb)
Mareeba—			
7	4·0	Yes	150·5
16	4·0	No	146·9
17	8·5	No	141·7
16	17·0	No	141·0
Burdekin—			
24	0·7	Yes	125·3
26	14·0	No	124·7

Because of its success in the 1959 season, zineb will be more widely used by tobacco growers in future, particularly by those in the Burdekin districts. However, it has been found (Pont 1959) that, under conditions of high inoculum potential, such as were experienced when wet weather led to a sudden intensification of the disease in a block of plants in which it had previously been confined mainly to the unsprayed plots, the protection afforded by regular zineb treatments was lost. Under these conditions a massive infection developed in plots which had been sprayed with this fungicide, and due to the resistance which unsprayed plants had acquired by virtue of their early infection, the leaf loss on the zineb-sprayed plants was greater than that on the untreated ones.

For this reason the known synergism of mixtures of salicylate and other organic fungicides (Graham *et al.* 1947) has been utilized in experimental work to augment the efficiency of zineb in an attempt to find a mixture which can be recommended without reservation to tobacco growers.

Mixtures containing zineb and methyl salicylate and zineb and benzyl salicylate which were found to be particularly effective in screening trials (Pont 1959) were used in field trials in 1958 and 1959 respectively at the Parada Tobacco Experiment Station. However, the late epiphytotics characteristic of the 1956 and 1957 seasons at this Station were not duplicated in these later years. The question of the relative efficiency of zineb alone and of these mixtures under field conditions extremely favourable to disease development has, therefore, not yet been answered.

VIII. SOME ASPECTS OF CONTROL

The foregoing discussion brings up certain aspects of the control programme which are considered of primary importance. These are briefly referred to here.

(a) Elimination of Sources of Inoculum

In North Queensland the important source of inoculum is the carryover of blue mould on the vast number of volunteer plants originating from seed shed from seed-heads on untopped plants or suckers. These seedlings are more of a problem when fields which have grown tobacco are fallowed or are sown to irrigated truck crops or other field crops such as cotton and peanuts. They seem to be less troublesome when the fields are not cultivated and grasses and weeds take over. Standover plants appear to play a minor role in the survival of the fungus, but nevertheless have to be considered.

Legislation was introduced in August 1959 in the form of an Amendment to the Regulations under the *Tobacco Industry Protection Act of* 1933, which gives Inspectors under the Act authority to order the destruction in a prescribed fashion of the following classes of plants:—

(1) Tobacco plants on an area of land on which harvesting has been completed, whether they be from the planted crop or regrowth from the planted crop or volunteers.

(2) Tobacco plants remaining in areas laid down as seedbeds and no longer required for transplanting into the field or not in a fit condition because of age or disease to be used for such a purpose.

(3) Tobacco plants growing outside an area or areas specifically planted for the production of tobacco seedlings or tobacco leaf.

The destruction of these sources of infection is incumbent on every tobacco grower. Individual efforts at eradication by conscientious growers delay the appearance of the disease but are of little eventual benefit unless all growers co-operate at the same time.

In the Burdekin districts the possibility of eradicating *Nicotiana glauca,* which appears to be ecologically confined to the levee soils, should be considered.

(b) Standardized Planting Dates

The legislation mentioned can be rendered ineffectual by the presence of out-of-season standing crops affected by mould. Off-season planting, that is, field planting in the autumn, is undesirable, particularly along the Burdekin River where tobacco farms are contiguous, since it provides large populations of overwintering plants on which, due to low temperatures and periods of drizzly rain, blue mould incidence is likely to be exceedingly high.

This trouble could be avoided by enforcing a standardized date for first planting. The eradication legislation could be strictly enforced for two months prior to this date to ensure a break in the life cycle of the fungus.

Strict enforcement of the existing legislation in conjunction with the standardization of planting dates would go a long way towards providing an escape from the disease.

(c) Use of Healthy Seedlings

The transplanting of mould-infected seedlings almost invariably leads to crop failure but more importantly establishes a concentrated source of inoculum early in the season. The use of the benzol vapour treatment as recommended will enable the planting of healthy seedlings. However, due to various causes, there is a shortage of seedlings on some farms in most years and there is usually an interchange of planting material when growers are forced to seek extra plants to make up deficiencies. The interchanged seedlings usually come from beds on which benzol treatment has been terminated and are very often infected. A certified source from which a grower could draw healthy seedlings as required would obviate indiscriminate traffic and planting of infected material.

IX. ACKNOWLEDGEMENT

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TOBACCO LEAF-PEST CONTROL INVESTIGATIONS, 1949-1955

By W. A. Smith, B.Sc.*

SUMMARY

During 1949-1955, 7 insecticide screening trials, 3 yield trials, 4 taint trials and one seedbed phytotoxicity trial were carried through.

The inefficiency of dusts, and the limitations of many insecticides for use in the control of tobacco pests, were demonstrated.

Endrin, and dieldrin at a greater concentration, were clearly the best materials for use on tobacco where the looper *Plusia argentifera* Gn. must be controlled. DDT was the most satisfactory against budworms, *Heliothis armigera* Hb. and *H. punctigera* Wallengr., and for the control of the cluster grub, *Prodenia litura* (F.). DDT, endrin and dieldrin as protective sprays were effective in controlling leaf-miner, *Gnorimoschema operculella* (Zell.).

I. INTRODUCTION

In 1948, tobacco leaf-pest control practices in Queensland included the use of lead arsenate on young plants as dust, spray or bait for the leaf-chewing noctuids (Smith 1952) and DDT for the leaf miner, *Gnorimoschema operculella* (Zell.). The expansion of irrigated areas in subsequent years was associated with consistent and destructive attacks by the tobacco looper, *Plusia argentifera* Gn., in particular in exploratory plots grown by agricultural officers in the Lower Burdekin district preliminary to general expansion. The reason for the increased pest status of the tobacco looper was the change permitted by irrigation from early summer to spring planting when more of the cropping season occurred during the period of maximum activity of the insect (Smith 1959).

Growers were faced with the difficulty of controlling the tobacco looper on large plants and well-established practices were not suitable for this purpose. The present paper deals with investigations of insecticidal control of the leaf pests, particularly *P. argentifera,* and covers pest kills, tainting and phytotoxicity.

II. MATERIALS

DDT.—An emulsion concentrate containing 20 per cent. w/v *p.p'* isomer in toluol.

A dispersible concentrate containing 50 per cent. w/v *p.p'* isomer in solubilizing agent.

A dispersible powder containing 50 per cent. w/w *p.p'* isomer.

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A dust containing 5 per cent. w/w *p.p'* isomer.

A dust containing 2 per cent. w/w *p.p'* isomer.

An emulsion concentrate containing 25 per cent. w/v *p.p'* isomer.

An emulsifiable concentrate containing 20 per cent. w/v *p.p'* isomer in aromatic (high range) solvent.

An emulsifiable concentrate containing 25 per cent. w/v *p.p'* isomer in solvent naphtha.

An emulsifiable concentrate containing 20 per cent. w/v *p.p'* isomer in benzol.

Lead arsenate.—A dispersible powder containing 31 per cent. w/w As_2O_5.

A dust containing 15·5 per cent. w/w As_2O_5.

Parathion.—An emulsifiable concentrate containing 20 per cent. w/v active ingredient in aromatic (high range) solvent.

A dispersible powder containing 15 per cent. w/w active ingredient.

An emulsifiable concentrate containing 25 per cent. w/v active ingredient in emulsifier.

Demeton.—An emulsifiable concentrate containing 50 per cent. w/v active ingredient in emulsifier.

TTC.—A dispersible powder containing 10 per cent. w/w bis (p-chlorophenoxy) methane.

White oil.—An emulsion concentrate containing 74 per cent. w/v refined mineral oil; unsulphonatable residue not less than 90 per cent.

BHC—An emulsifiable concentrate containing 7 per cent. w/v gamma isomer and other isomers in mineral turpentine.

An emulsifiable concentrate containing 16 per cent. w/v gamma isomer (lindane) in toluol.

A dispersible powder containing 16 per cent. w/w gamma isomer (lindane).

An emulsifiable concentrate containing 14 per cent. w/v gamma isomer (lindane).

Toxaphene.—An emulsifiable concentrate containing 60 per cent w/v chlorinated camphenes.

An emulsifiable concentrate containing 100 per cent. w/v chlorinated camphenes.

Dieldrin.—An emulsifiable concentrate containing 16 per cent. w/v active ingredient.

An emulsifiable concentrate containing 15 per cent. w/v active ingredient in aromatic (low range) solvent.

A dispersible powder containing 50 per cent. w/w active ingredient.

A dust containing 5 per cent. w/w active ingredient.

An emulsifiable concentrate containing 20 per cent. w/v active ingredient in solvent naphtha.

Mipafox.—A miscible solution containing 50 per cent. w/v active ingredient (anhydrous).

Pyrethrum/piperonyl butoxide.—A dust containing 0·05 per cent. w/w pyrethrins and 0·8 per cent. w/w piperonyl butoxide.

Pyrethrum/piperonyl butoxide/DDT.—A dust containing 0·048 per cent. w/w pyrethrins, 0·8 per cent. w/w piperonyl butoxide and 2 per cent. w/w. *p.p'* isomer of DDT.

Chlordane.—An emulsifiable concentrate containing 82 per cent. w/v active ingredient in deodorized kerosene.

Endrin.—An emulsifiable concentrate containing 20 per cent. w/v active ingredient in aromatic (low range) solvent.

An emulsifiable concentrate containing 18·5 per cent. w/v active ingredient.

DDD.—An emulsion concentrate containing 20 per cent. w/v active ingredient in solvent naphtha.

Aldrin.—An emulsifiable concentrate containing 23 per cent. w/v active ingredient.

Aromatic (high range) solvent.—A solvent containing 95 per cent. v/v aromatics, having a distillation range 138°-177° C.

Aromatic (low range) solvent.—A solvent containing 95 per cent. v/v aromatics, having a distillation range 112°-162° C.

Solvent naphtha.—A high commercial grade.

Emulsifier.—A commercial sodium lauryl sulphonate.

Benzol.—A commercial grade.

Toluol.—A commercial grade.

III. PEST KILLS

Seven screening trials and 3 yield trials were carried through.

(a) Methods

(i) Screening Trials

Sites were selected in established plantings where loopers were active, or crops were grown for the special purpose, receiving sufficient insecticidal protection in the establishment period to allow growth to proceed in the presence of other pests.

A medium-sized plant in active growth was aimed at for trial purposes to ensure attractiveness to larvae and moths and to avoid the increased parasite activity often found on older plants. All trials were furrow irrigated.

The plot size was a definite number of plants in each trial, varying from 2 to 5 in different trials, depending on the number of larvae present per plant. The method of varying the plot size to give a minimum number of larvae per plot prior to treatment was abandoned after an unsuccessful attempt during 1952 to determine synergistic effects between DDT and lead arsenate. Oviposition during the post-treatment period resulted in disproportionate population increases due to the variable plot size.

Insecticides were applied with a knapsack sprayer or a small hand dust gun. Special efforts were made to obtain a complete cover on both sides of each leaf. In most trials framed screens of hessian were used to restrict sprays and dusts.

Loopers at all stages were counted on each plot the day prior to treatment and again at intervals up to 14 days after treatment. The technique of preparing the plant for the pre-treatment count varied with its nature. If necessary, the inflorescence and axillary branches were removed and the area was cleared of weeds.

Details of layout, plot size, treatments and other relevant information are given with the results.

(ii) Yield Trials

Only sprays by knapsack sprayer and dry baits by hand were used.

Five random plants per plot were used for insect counts in these trials with the following exceptions. Ten plants were used for the first 3 counts in the 1950 and the 1953 trials when the plants were small; 12 plants were used to represent each control strip in the 1953 and the 1954 trials.

In addition to loopers, the larvae of *Heliothis* species (*H. armigera* Hb. and *H. punctigera* Wallengr.) and *Prodenia litura* F., and the mines of the leaf-miner, were counted. In the 1950 trial the pupal clusters of a Braconid, commonly parasitic on *P. argentifera* larvae, were also recorded.

The percentage of leaf area undamaged was visually estimated a week prior to the beginning of harvests in the 1950 trial. The leaves of 5 random plants were used, the numbers ranging from 78 to 100 per plot.

As the crop ripened, plots were harvested separately and labelled sticks of leaf were cured and afterwards bulked as parcels. When ready for grading, the parcels were weighed and recorded by plots. Samples of each grade were given an assessed value by a trade appraiser and plot values were calculated accordingly.

(b) Results

(i) Screening Trials, 1949

Two trials were carried through at Claredale during December 9-16. In both trials the layout was a 14 x 4 randomized block with plot sizes of 3 plants (Trial 1) and 2 plants (Trial 2). Plants were 2-3 ft high in Trial 1 and 6-7 ft in Trial 2. Results are presented in Table 1 as mean survival indices, calculated by dividing numbers of larvae surviving by numbers before treatment. Mean numbers (and range) of larvae per plot prior to treatment were 35·2 (16-72) in Trial 1 and 21·6 (8-48) in Trial 2.

Table 1

MEAN SURVIVAL OF *Plusia argentifera*

(*Screening Trials, Claredale*, 1949)

Treatment		Survival Indices			
		Trial 1		Trial 2	
		After 3 Days	After 7 Days	After 3 Days	After 7 Days
DDT toluol emulsion 0·2%		0·22	0·10	0·08	0·08
DDT toluol emulsion 0·1%		0·28	0·18	0·09	0·12
DDT solubilized spray 0·3%		0·42	0·49	0·08	0·12
DDT dispersible powder 0·4%		0·62	0·46	0·12	0·12
DDT dispersible powder 0·2% plus white oil 1 : 200		0·50	0·50	0·03	0·03
DDT dust 5%		0·99	1·14	0·47	0·28
DDT dust 2%		0·98	0·83	0·13	0·23
Lead arsenate spray 3 lb / 50 gal		0·08	0·03	0·01	0·00
Lead arsenate dust		0·54	0·38	0·09	0·08
DDT toluol emulsion 0·2% plus lead arsenate 1½ lb / 50 gal		0·32	0·23	0·00	0·01
Parathion aromatic (high range) emulsion 0·05%		0·07	0·26	0·03	0·01
Parathion aromatic (high range) emulsion 0·01%		0·22	0·50	0·12	0·10
DDT toluol emulsion 0·4% plus lead arsenate 3 lb / 50 gal		0·10	0·05	..	..
Untreated		..	..	0·44	0·34
Untreated		1·96	2·51	0·58	0·57
Necessary differences for significance—					
All treatments	5%	0·56	0·66	0·24	0·22
	1%	0·75	0·88	0·32	0·29
Excluding 6th, 7th and 14th	5%	0·25	0·27	..	..
	1%	0·34	0·36	..	..
Excluding 6th, 13th and 14th	5%	..	..	0·11	..
	1%	..	..	0·15	..
Excluding 6th, 7th, 13th and 14th	5%	..	..	..	0·12
	1%	..	..	..	0·16

The figures demonstrate the efficacy of DDT and lead arsenate and the superiority of sprays over dusts. In Trial 1 the population increase due to egg hatching provided conditions which revealed the reduction in residual activity of parathion within 7 days of treatment.

(ii) Screening Trials, 1951

Two trials were carried through between November 29 and December 17, one at Kalamia and one at Claredale. The respective layouts were 13 x 4 and 14 x 4 randomized blocks with plot sizes of 3 plants 4-6 ft high. Mean numbers (and ranges) of loopers per plot before treatment were 28·8 (15-73) at Kalamia and 22·0 (15-75) at Claredale. To indicate population fluctuations, percentage natural survival is given in Table 2. An adjustment was made in the observed percentage survival figures to allow comparisons from day to day. The percentages on each day were multiplied by the factor necessary to bring the untreated plot survival percentages to 100. The adjusted percentages are presented in Table 3.

Table 2

PERCENTAGE NATURAL SURVIVAL

(*Screening Trials, Kalamia and Claredale*, 1951)

Trial	After 1 Day	After 3 Days	After 7 Days	After 11 Days	After 13 Days
Kalamia ..	112	89	118	249	..
Claredale ..	97	52	49	..	45

Table 3

MEAN ADJUSTED PERCENTAGE SURVIVAL

(*Screening Trials, Kalamia and Claredale*, 1951)

Treatment	Kalamia				Claredale			
	After 1 Day	After 3 Days	After 7 Days	After 11 Days	After 1 Day	After 3 Days	After 7 Days	After 13 Days
DDT toluol emulsion 0·1%	39	37	43	65	39	33	32	92
DDT toluol emulsion 0·1% plus lead arsenate 3 lb / 50 gal	47	25	14	46	42	17	..	12
Lead arsenate spray 3 lb / 50 gal plus white oil 1 : 250	83	47	30	65	64	20	22	40
BHC emulsion 0·03% with other isomers	68	14	45	143	21	6	59	192
BHC emulsion 0·03% with other isomers plus DDT toluol emulsion 0·1%	44	9	32	91	10	2	7	90
Demeton emulsion 0·025%	98	105	73	100	..	..	..	..
Toxaphene 60% emulsion 0·1% ..	..	..	..	..	19	4	6	44
Dieldrin 16% emulsion 0·05% ..	44	11	16	49	30	6	5	27
Dieldrin 16% emulsion 0·1%	39	4	2	17	5	2	1	1
Demeton emulsion 0·075%	84	91	90	130	88	82	99	175
Mipafox spray 0·05%	76	109	102	86	77	92	100	136
Pyrethrum dust 0·05% plus piperonyl butoxide 0·8%	33	31	72	87	68	101	150	145
Pyrethrum dust 0·048% plus piperonyl butoxide 0·8% plus DDT 2% ..	57	61	76	103	84	85	91	60
Chlordane emulsion 0·1%	..	..	..	..	45	33	62	83
Untreated	100	100	100	100	100	100	100	100
Necessary differences for significance 5%	26	32	44	52	20	33	76	111
Necessary differences for significance 1%	35	43	59	70	26	44	102	148

Dieldrin was efficacious in both initial and residual toxicity. Toxaphene was promising. BHC had high toxicity but brief residual life. Chlordane produced moderate kills, but pyrethrum, mipafox and parathion gave no promise in these two trials. Observations during counting indicated that lead arsenate, more than other insecticides, killed larvae slowly, permitting some which were incapable of feeding to be included in the count.

(iii) Screening Trial, 1953

This was carried through at Claredale during December 4-23. The layout was a 17 x 4 randomized block with plots of 5 tall plants. Results are presented in Table 4 as percentage survival of loopers 1 day after treatment. Few remained on any except untreated plots after 3 days. Mean of plot counts (and range) prior to treatment was 41·2 (30-63).

Table 4

PERCENTAGE SURVIVAL ONE DAY AFTER TREATMENT

(*Screening Trial, Claredale*, 1953)

Treatment	Transformed Mean*	Equivalent Mean (%)
Endrin 20% emulsion 0·05%	3·1	0·3
Endrin 20% emulsion 0·025%	14·3	6·1
Endrin 20% emulsion 0·0125%	25·0	17·9
Dieldrin aromatic (low range) emulsion 0·05%	15·3	7·0
Dieldrin dispersible powder 0·05%	28·8	23·2
Dieldrin dust 5%	26·6	20·1
Parathion aromatic (high range) emulsion 0·03%	16·1	7·7
Parathion aromatic (high range) emulsion 0·02%	23·5	15·9
Parathion aromatic (high range) emulsion 0·01%	32·7	29·2
Parathion dispersible powder 0·03%	21·1	13·0
Parathion dispersible powder 0·02%	19·0	10·6
Parathion dispersible powder 0·01%	25·2	18·1
BHC dispersible powder 0·03%	18·8	10·4
BHC toluol emulsion 0·03%	32·3	28·6
DDT toluol emulsion 0·1% plus lead arsenate 3 lb / 50 gal	34·2	31·6
Untreated (2 plots)	(83·7)	98·8
Necessary differences for significance 5%	7·14	..
Necessary differences for significance 1%	9·63	..

* Inverse sine.

Endrin was the best insecticide at practical levels, achieving a large kill after 1 day compared with the DDT plus lead arsenate spray adopted as a standard.

(iv) Screening Trials, 1955

Two trials were carried through, at Claredale beginning on November 16 and at Ayr beginning on November 25, with 6 x 4 randomized block layouts. Plots of 8 tall plants were treated and 3 middle plants were used for counts. Results of counts of *P. argentifera* larvae are given in Table 5 (Claredale) and Table 7

(Ayr) as mean numbers per plant. *Heliothis* species were also present and similar data recorded concerning these is presented in Table 6 (Claredale) and Table 8 (Ayr). Natural population depression limited the number of counts following treatment.

Table 5

MEAN NUMBERS OF *Plusia argentifera* LARVAE

(*Screening Trial, Claredale*, 1955)

Treatment	Pretreatment		After 1 Day		After 3 Days	
	Trans-formed Mean*	Equiv. Mean No.	Trans-formed Mean*	Equiv. Mean No.	Trans-formed Mean*	Equiv. Mean No.
DDD emulsion 0·05%	2·42	5·36	1·46	1·63	1·47	1·66
DDD emulsion 0·1%	3·28	10·26	1·61	2·09	1·06	0·62
DDD emulsion 0·2%	3·09	9·05	1·76	2·60	0·96	0·42
Endrin 20% emulsion 0·05%	3·05	8·80	0·84	0·21	0·71	0·00
Dieldrin aromatic (low range) emulsion 0·05%	2·89	7·85	1·31	1·22	0·84	0·21
Untreated	2·48	5·65	2·03	3·62	1·62	2·12
Necessary differences for significance 5%	1·55	..	0·86	..	0·47	..
Necessary differences for significance 1%	2·14	..	1·19	..	0·65	..

* $\sqrt{x + \frac{1}{2}}$

Table 6

MEAN NUMBERS OF LARVAE OF *Heliothis* SPECIES

(*Screening Trial, Claredale*, 1955)

Treatment	Pretreatment		After 1 Day		After 3 Days		After 7 Days	
	Trans-formed Mean*	Equiv. Mean No.	Trans-formed Mean*	Equiv. Mean No.	Trans-formed Mean*	Equiv. Mean No.	Trans-formed Mean*	Equiv. Mean No.
DDD emulsion 0·05%	2·38	5·16	1·34	1·30	0·96	0·42	1·38	1·40
DDD emulsion 0·1%	1·72	2·46	1·19	0·92	0·96	0·42	2·28	4·70
DDD emulsion 0·2%	2·36	5·07	0·96	0·42	0·71	0·00	0·71	0·00
Endrin 20% emulsion 0·05%	2·24	4·52	1·26	1·09	1·26	1·09	2·12	3·99
Dieldrin aromatic (low range) emulsion 0·05%	2·70	6·79	1·68	2·32	0·93	0·36	0·84	0·21
Untreated	2·59	6·21	2·71	6·84	3·19	9·68	3·89	14·63
Necessary differences for significance 5%	1·07	..	0·92	..	0·69	..	1·97	..
Necessary differences for significance 1%	1·48	..	1·27	..	0·96	..	2·73	..

* $\sqrt{x + \frac{1}{2}}$

Table 7

MEAN NUMBERS OF *Plusia argentifera* LARVAE

(*Screening Trial, Ayr*, 1955)

Treatment	Pretreatment		After 1 Day	
	Transformed Mean*	Equiv. Mean No.	Transformed Mean*	Equiv. Mean No.
DDD emulsion 0·05%	3·19	9·68	2·47	5·60
DDD emulsion 0·1%	3·61	12·53	2·11	3·95
DDD emulsion 0·2%	3·70	13·19	1·46	1·63
Endrin 20% emulsion 0·05%	4·29	17·90	0·93	0·36
Dieldrin aromatic (low range) emulsion 0·05%	2·71	6·84	1·26	1·09
Untreated	4·11	16·39	3·60	12·46
Necessary difference for 5% significance	1·76	..	0·83	..
Necessary difference for 1% significance	2·43	..	1·15	..

* $\sqrt{x + \frac{1}{2}}$

Table 8

MEAN NUMBERS OF LARVAE OF *Heliothis* SPECIES

(*Screening Trial, Ayr*, 1955)

Treatment	Pretreatment		After 1 Day		After 3 Days		After 7 Days	
	Transformed Mean*	Equiv. Mean No.	Transformed Mean*	Equiv. Mean No.	Transformed Mean*	Equiv. Mean No.	Transformed Mean*	Equiv. Mean No.
DDD emulsion 0·05%	4·46	19·39	1·42	1·52	1·06	0·62	1·82	2·81
DDD emulsion 0·1%	5·09	25·41	1·44	1·57	0·84	0·21	1·56	1·93
DDD emulsion 0·2%	4·88	23·31	1·34	1·30	0·84	0·21	1·13	0·78
Endrin 20% emulsion 0·05%	5·10	25·51	2·64	6·47	1·60	2·06	1·99	3·46
Dieldrin aromatic (low range) emulsion 0·05%	5·80	33·14	2·93	8·08	1·31	1·22	2·56	6·05
Untreated	5·22	26·75	4·85	23·02	3·46	11·47	4·30	17·99
Necessary differences for 5% significance	1·53	..	1·17	..	1·08	..	1·27	..
Necessary differences for 1% significance	2·11	..	1·62	..	1·50	..	1·76	..

* $\sqrt{x + \frac{1}{2}}$

Despite small populations, the results, using dieldrin as a new standard, suggest that DDD has only moderate toxicity to loopers and is little better than dieldrin and endrin against *Heliothis* species at normal levels.

(v) Yield Trial, 1950

The yield trial carried through at Claredale in 1950 was the first of its type in these investigations. Leaf was harvested on 4 days between November 28 and December 21. The 6 x 4 layout included an untreated plot as one of

the randomized treatments. Plots were 6 rows x 57 ft with 4 rows x 57 ft (0·01 ac) as data area. Details of materials and timing of treatment programmes are set out in Table 9; the DDT formulation used was the toluol emulsion. Results of yields, leaf value and leaf damage are given in Table 10 with looper populations in November; counts on September 28 and October 9 rarely exceeded 0·15 per plant. Data on populations of parasites and pests other than the tobacco looper are presented in Table 11.

Table 9

TREATMENT PROGRAMMES

(*Yield Trial, Claredale*, 1950)

Programme	28–ix–50	5–x–50	24–x–50	15–xi 50
1	DDT spray 0·1%	DDT spray 0·1%	DDT spray 0·1%	DDT spray 0·1%
2	DDT spray 0·2%	DDT spray 0·2%	DDT spray 0·2%	DDT spray 0·2%
3	DDT spray 0·2% plus lead arsenate 1½ lb /50 gal	DDT spray 0·2% plus lead arsenate 1½ lb /50 gal	DDT spray 0·2% plus lead arsenate 1½ lb /50 gal	Lead arsenate spray 1½ lb/50 gal
4	As 3	As 3	Lead arsenate spray 3 lb / 50 gal	Bran bait containing 5% lead arsenate
5	As 3	As 3	As 3	DDT spray 0·2% plus lead arsenate 1½ lb / 50 gal
6	Nil	Nil	Nil	Nil

Table 10

YIELDS, APPRAISED VALUES, LOOPER COUNTS AND LEAF DAMAGE

(*Yield Trial, Claredale*, 1950)

Programme	Mean Yield (lb/ac)	Appraised Value (£/ac)	Immature Loopers (Insects/Plant)		Index of Leaf Area Undamaged 22.xi.50 (Max. area = 100)
			7.xi.50	22.xi.50	
1	1132·5	377·0	1·48	2·65	92·8
2	1049·0	340·2	2·98	2·25	91·0
3	1007·5	311·8	2·12	1·70	94·5
4	965·5	330·2	1·90	3·00	90·2
5	1126·8	368·5	2·40	2·40	90·0
6	824·5	261·5	1·35	2·10	83·5
Necessary differences for significance 5%	216·5	96·5	1·02	2·18	.. 5·5
Necessary differences for significance 1%	299·4	133·4	1·41	3·01	7·6

Table 11

OTHER PEST AND PARASITE POPULATIONS

(*Yield Trial, Claredale*, 1950)

Programme	Numbers/Plant						
	11. ix. 50	7. xi. 50			22. xi. 50		
	G. operculella Leaf Mines	*Heliothis* Species Larvae	*Prodenia litura* Larvae	Braconid Pupal Clusters	*Heliothis* Species Larvae	*Prodenia litura* Larvae	Braconid Pupal Clusters
1	0	0·075	0·075*	0·025	0	0·05	0·50
2	0	0	0·05 *	0	0	0	0·55
3	0·0125	0·025	0·125	0·025	0·65	0·50	0·15
4	0·0125	0	0·125*	0	0·30	0·60	0·45
5	0	0·075	0·15**	0	0·05	0·05	0·35
6	0·94	0·325	0·025	0·05	0·20	0·80	0·60

* These plots had 1 cluster of larvae per plant in addition to the solitary larvae counted.

All the spray programmes were of value in protecting leaves in this trial. Pest populations were small but persistent and untreated plants suffered a distinct though small loss of yield. The numbers of Braconid pupal clusters indicate moderate parasitism of *P. argentifera* during November and may explain the relative smallness of populations of that pest.

(vi) Yield Trial, 1953

This trial was carried through at Claredale between early September, 1953, and early January, 1954. The layout was a 4 x 6 randomized block with 4 control strips in place of randomized control plots. Treatment plots were 8 rows x 35 ft with a data area 6 rows x 33 ft (0·018 ac); control strips were 32 rows x 13 ft with a data area 24 rows x 11 ft (0·024 ac). Details of materials and timing of treatment programmes are set out in Table 12; the insecticide formulations were DDT toluol emulsion and dieldrin aromatic (low range) emulsion. Results of yields and values are presented in Table 13. Periodic insect counts are set out in Figures 1-4: the major differences are statistically sound in all instances.

At the frequency used, both dieldrin and DDT satisfactorily protected the crop from the pest complex demonstrated in the graphs. Untreated plants were badly damaged. In the graphs the superiority of DDT over dieldrin for *Heliothis* control, and of dieldrin over DDT for looper control, is apparent. Both prevented leaf-miner attack.

Table 12

TREATMENT PROGRAMMES

(*Yield Trial, Claredale*, 1953)

Date	Programme			
	1	2	3	4
17. ix. 53	DDT spray 0·1%	Dieldrin spray 0·05%	DDT spray 0·1% plus lead arsenate 3 lb/50 gal	DDT spray 0·1%
24. ix. 53	DDT spray 0·1%	Dieldrin spray 0·05%	DDT spray 0·1% plus lead arsenate 3 lb/50 gal	DDT spray 0·1%
10. x. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%
12. x. 53	..	..	Bran bait containing 5% lead arsenate	..
22–23. x. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%
29–30. x. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%
9–10. xi. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%
24. xi. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%
1. xii. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%
22. xii. 53	Dieldrin spray 0·05%	Dieldrin spray 0·05%	DDT spray 0·1%	DDT spray 0·1%

Table 13

YIELDS AND APPRAISED VALUES

(*Yield Trial, Claredale*, 1953)

Programme	Cured Leaf	
	Mean Yield (lb/ac)	Appraised Value (£/ac)
1	1523	781
2	1549	788
3	1576	784
4	1475	746
Untreated	(276)	(117)
Necessary differences for significance 5%	150	61
Necessary differences for significance 1%	208	85

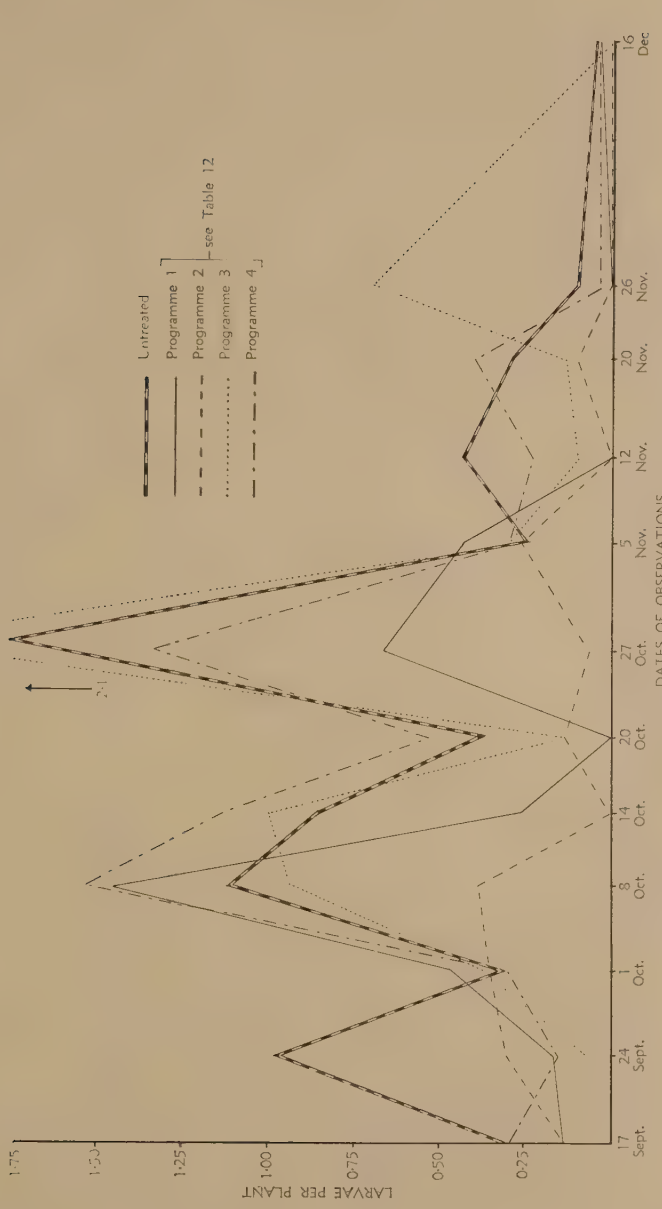

Fig. 1.—Yield trial, 1953. Suppression of *Plusia argentifera*. Dieldrin (Programmes 1 and 2) was more efficacious than DDT (Programmes 3 and 4).

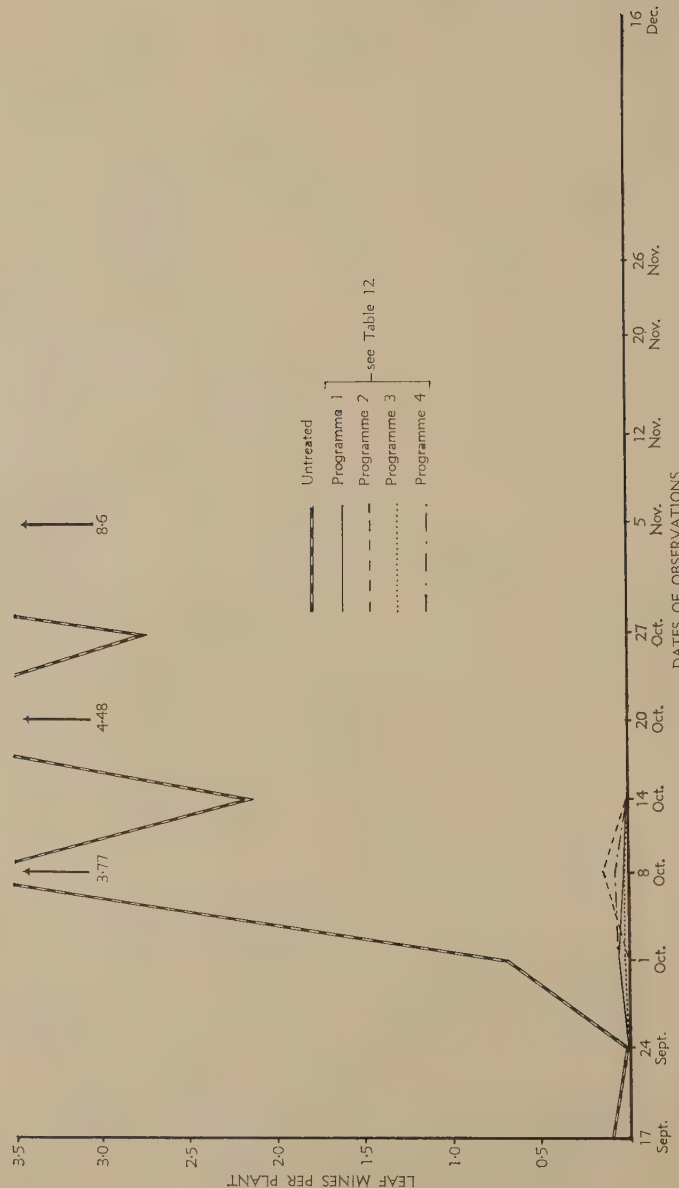

Fig. 2.—Yield trial, 1953. Suppression of *Gnorimoschema operculella*. All programmes were efficacious.

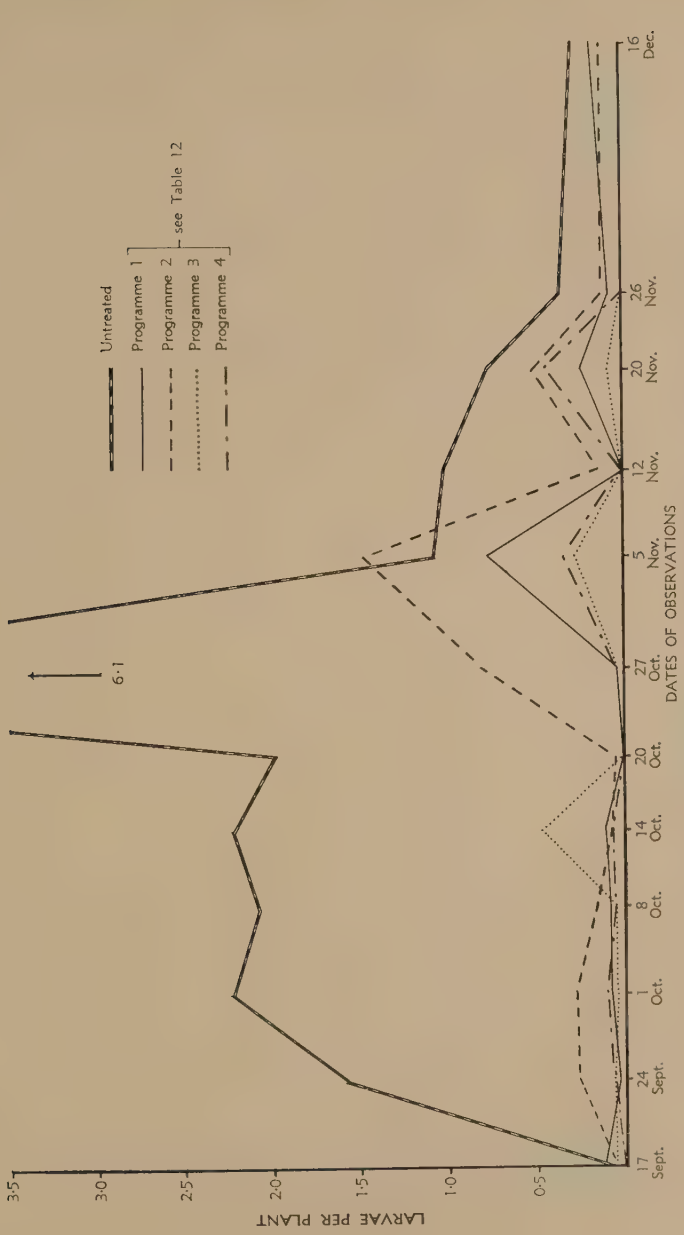

Fig. 3.—Yield trial, 1953. Suppression of *Heliothis* species. DDT (Programmes 3 and 4) was more efficacious than dieldrin (Programmes 1 and 2).

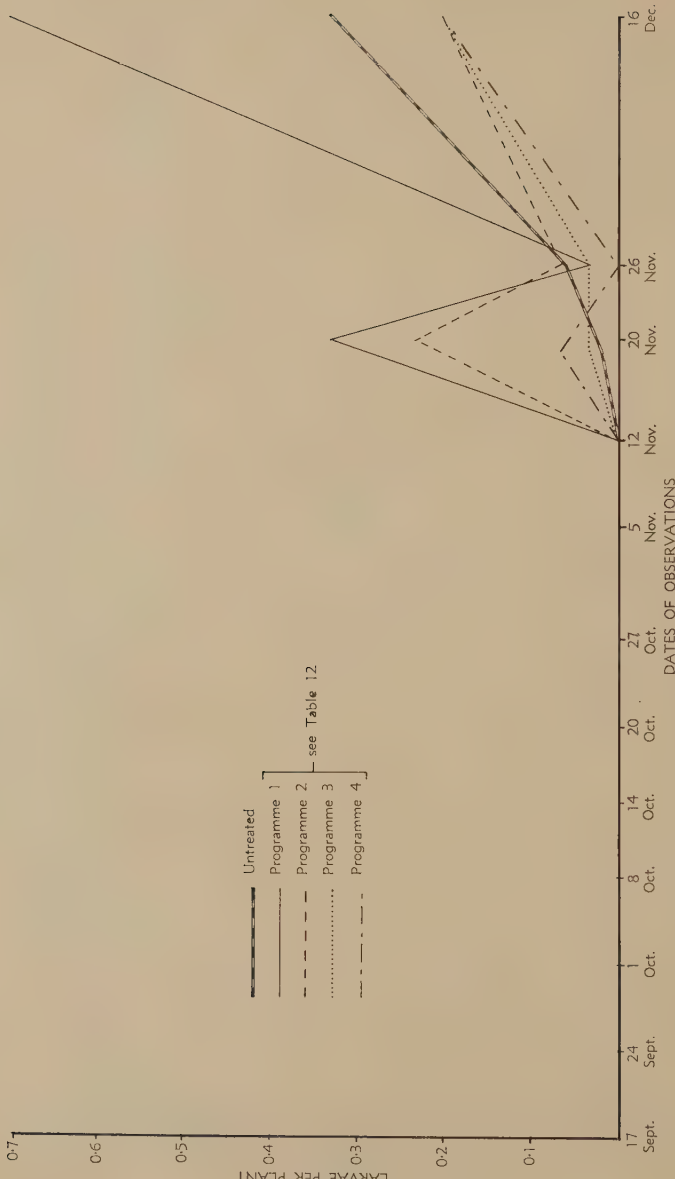

Fig. 4.—Yield trial, 1953. Suppression of *Prodenia litura*. DDT (Programmes 3 and 4) was more efficacious than dieldrin (Programmes 1 and 2).

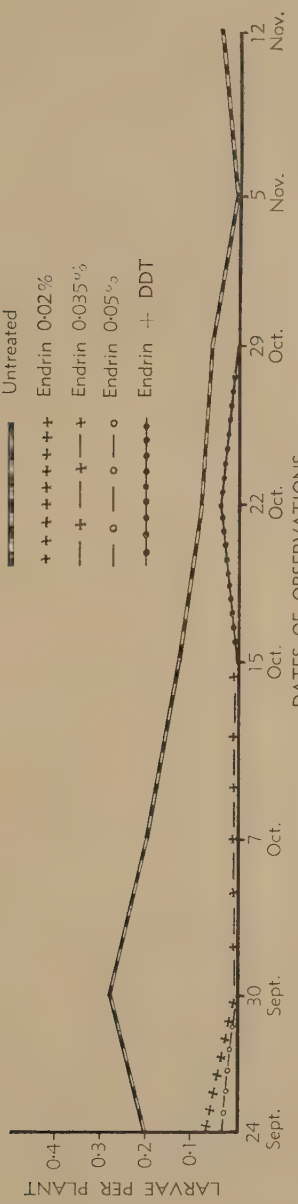

Fig. 5.—Yield trial, 1954. Suppression of *Plusia argentifera*.

(vii) Yield Trial, 1954

This trial was begun at Claredale in September 1954, and terminated prematurely in November when storms destroyed a high percentage of plants previously weakened by blue mould disease. The layout was a 4 x 6 randomized block with control strips. Treatment plots were 6 rows x 35 ft with a data area of 4 rows x 33¼ ft (0·012 ac); control strips were 24 rows x 14 ft with a data area of 20 rows x 12¼ ft (0·023 ac).

The 4 treatments were endrin aromatic (low range) emulsion at 0·02, 0·035 and 0·05 per cent. levels and the 0·02 per cent. level plus DDT toluol emulsion 0·1 per cent. All plots were sprayed on September 16, September 24, September 30, October 8, October 22, November 1 and November 11. Results of pest counts during the treatment period are presented in Figures 5-7.

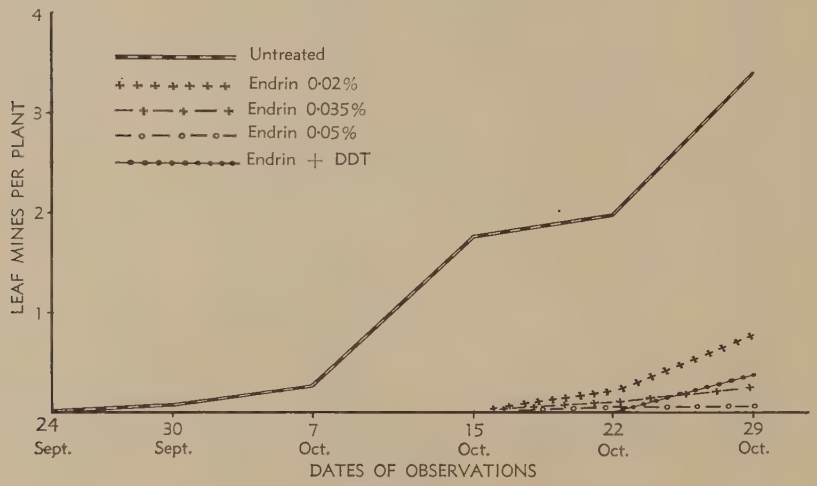

Fig. 6.—Yield trial, 1954. Suppression of *Gnorimoschema operculella.*

With small pest populations endrin effectively controlled the tobacco looper. It was inferior to DDT for control of *Heliothis* species but equal to it in controlling leaf-miner.

(c) Discussion

The screening of numerous insecticides against the tobacco looper and their testing on a field scale against the pest complex of tobacco permitted periodic recommendations of efficacious materials. When dieldrin, and later endrin, became available these proved outstanding in percentage kill and persistence. *Heliothis* species were not controlled well enough by dieldrin or endrin and the value of retaining DDT for that purpose was demonstrated by trial results. Under the conditions of the trials all three materials were effective protective sprays against leaf-miner.

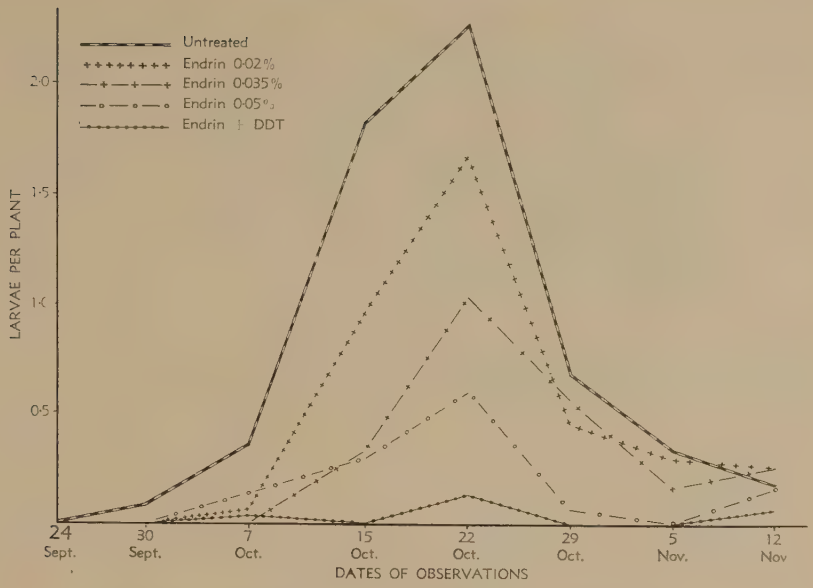

Fig. 7.—Yield trial, 1954. Suppression of *Heliothis* species.

The shortcomings of dusts and the failure of insecticides with short residual life under conditions of increasing pest populations in this work resolved doubts on a number of insecticides and formulations.

IV. TAINTING OF LEAF

Materials of possible commercial value were taken through taint tests during 1952-1954.

(a) Methods

Insecticides at commercial strengths and rates were sprayed by knapsack on plants in the field. Numbered samples of the cured leaf, 3 lb in weight where possible, were separately wrapped in "Cellophane" and submitted to a panel of trade assessors, which reported on any taints associated with each number. From these reports the results of each trial were prepared from the keys held.

(b) Results

(i) Taint Trial, 1952

Plants at Claredale were sprayed once during January and harvested a week later. Nine samples of cured leaf were prepared from each unreplicated treatment plot. The results are presented in Table 14.

Table 14

TAINT TRIAL, CLAREDALE, 1952

Treatment	No. of Samples		
	Distinctly Tainted	Slightly Tainted	Not Tainted
Toxaphene 60% emulsion 0·1% ..	4	0	4
Chlordane emulsion 0·1%	8	0	1
Dieldrin 16% emulsion 0·05%	0	1	8
BHC emulsion 0·03% with other isomers	8	0	1
Aldrin emulsion 0·1%	0	1	8
Untreated	0	0	9

One late spraying with chlordane, BHC mixed isomers or toxaphene was sufficient to taint the cured leaf.

(ii) Taint Trial, Tiaro, 1953

Leaf was sprayed once and harvested a week later at Tiaro during January. The field layout was a 12 by 3 randomized block with one 1 lb sample taken from each plot. Results are presented in Table 15.

Table 15

TAINT TRIAL, TIARO, 1953

Treatment	No. of Samples		
	Distinctly Tainted	Slightly Tainted	Not Tainted
Dieldrin 16% emulsion 0·05%	0	4	2
BHC 14% emulsion 0·03%	0	3	0
BHC emulsion 0·03% with other isomers	3	0	0
Toxaphene 100% emulsion 0·1% ..	3	0	0
Aldrin emulsion 0·1%	0	3	0
Chlordane emulsion 0·1%	0	3	0
Endrin 18·5% emulsion 0·1%	1	2	0
Parathion 25% emulsion 0·01% ..	0	1	2
TTC dispersible powder 0·1%	3	0	0
DDT toluol emulsion 0·1% plus lead arsenate 3 lb / 50 gal	0	3	0
Untreated	0	3	0

Careful mapping of the trial area and adjacent areas for ground irregularities yielded an explanation for the unexpected occurrence of slight, and even distinct, taints. These plots were in lowlying parts of the field and were unhealthy in appearance; root-knot nematodes were present in some. Tainting can therefore be a function of leaf quality. BHC mixed isomers, toxaphene and TTC were the only materials giving a distinct taint consistently.

(iii) Taint Trial, Claredale, 1953

This trial at Claredale was located on tobacco grown from September 11, 1953, to January 6, 1954. The layout was a 4 x 3 randomized block with plots of 126 plants. From time of planting out plots were sprayed 14 times with the test materials only. Two samples of cured leaf were taken from each plot, representing early and late harvests. Nematode indices of plant roots after harvesting were allotted to plots using a method previously referred to (Smith 1957). Results are presented in Table 16.

Table 16

TAINT TRIAL, CLAREDALE, 1953

Treatment	Harvest	No. of Samples			Nematode Index (Max. Infestation = 100)
		Distinctly Tainted	Slightly Tainted	Not Tainted	
Endrin 18·5% emulsion 0·05% ..	Early	0	2	1	62
	Late	0	1	2	
BHC toluol emulsion 0·03%	Early	2	1	0	54
	Late	0	2	1	
Dieldrin aromatic (low range) emulsion 0·05%	Early	0	1	2	52
	Late	0	1	2	
DDT toluol emulsion 0·1%	Early	0	3	0	58
	Late	0	2	1	

BHC failed to protect the plants from pests sufficiently to yield large samples of cured leaf. Distinct tainting was reported in two small samples. Leaf quality throughout was not high and this no doubt led to assessment of slight tainting.

(iv) Taint Trial, 1954

Unreplicated plots of 200 tobacco plants were sprayed twice at Inglewood during April. Two harvests, each three days after a spray application, provided cured leaf from which 2 lb samples were taken. Results are presented in Table 17.

Table 17

TAINT TRIAL, INGLEWOOD, 1954

Treatment	No. of Samples		
	Distinctly Tainted	Slightly Tainted	Not Tainted
Endrin 18·5% emulsion 0·025% ..	0	2	1
Endrin 18·5% emulsion 0·05% ..	0	1	2
Dieldrin aromatic (low range) emulsion 0·05%	0	1	2
BHC emulsion 0·033%	2	0	0
Untreated	1	2	0

Only BHC gave a consistent, distinct taint. Leaf quality in the block was affected by cyclone damage, insect damage and slow ripening during cold weather.

(c) Discussion

Leaf classed as distinctly tainted was defined as objectionable when used alone or in blends. Slight tainting implied the presence of undesirable flavours which could be masked by blending the leaf. The evidence indicates that trace flavours introduced by insecticides could not be separated from flavours due to other factors.

In these trials the insecticides toxaphene, chlordane, BHC and TTC produced objectionable taints in cured leaf when sprayed on plants in the field close to harvesting. Endrin, dieldrin, aldrin, parathion and DDT did not cause taints separable from those due to growth and curing conditions.

Three facts apparent in this work and of value in future taint trials on tobacco are:—(1) leaf quality is an important factor in the detection of taints, and it is essential to use only first-quality crops; (2) provision should be made to ensure that 3 lb of leaf will be available for each sample; and (3) BHC as mixed isomers has emerged as a reliable standard for positive taint.

V. PHYTOTOXICITY OF INSECTICIDES

The phytotoxicity of common formulations of DDT and dieldrin was tested in 1953.

(a) Methods

Seedlings were grown at Claredale by standard methods and sprayed or dusted at intervals with the test materials until large enough to plant out in the field. Beds were seeded on September 28 and treated on October 25, October 31 and November 7. The layout was a 16 x 4 randomized block with a 2 ft x 1 ft data area in each 4 ft x 1¼ ft plot. The materials were applied with a 1 pint continuous pressure hand atomizer and a small dust gun, while a 3-sided screen restricted the materials to the plot. During November 16–18 the seedlings were dug out, washed and counted, and subsequently dried for 72 hr at 140° F and weighed.

(b) Results

Results are presented in Table 18.

(c) Discussion

DDT in solvent naphtha caused stunting of seedlings, which recovered slowly from obvious burning by the first two applications. Solvent naphtha alone caused no damage and some other formulations of DDT caused none.

DDT dust caused basal chlorosis and stunting of leaves with "dishing" and later recovery. This is consistent with injury observed on the young growth of field plants after dusting with DDT.

The variability within the trial allows only the conclusion that no gross reduction in seedling size was produced.

Table 18

MEAN MOISTURE-FREE SEEDLING WEIGHT

(*Phytotoxicity Trial, Claredale*)

Treatment	Weight/Plant (micrograms)
Solvent naphtha emulsion 1 : 200	301
Dieldrin solvent naphtha emulsion 0·1%	284
Untreated	277
Aromatic (high range) solvent emulsion 1 : 200	274
DDT aromatic (high range) emulsion 0·1%	255
Dieldrin dispersible powder 0·05%	248
Emulsifier 1 : 200	244
Dieldrin dust 5%	243
DDT dispersible powder 0·1%	225
DDT benzol emulsion 0·1%	223
DDT toluol emulsion 0·1%	222
Toluol emulsion 1 : 200	220
Dieldrin aromatic (low range) emulsion 0·05%	215
DDT solvent naphtha emulsion 0·1%	207
Benzol emulsion 1 : 200	202
DDT dust 2%	183
Necessary differences for significance 5%	64
Necessary differences for significance 1%	86

VI. GENERAL DISCUSSION AND CONCLUSIONS

The three phases of this investigation clearly point to endrin as the best of the materials available for use on tobacco where *P. argentifera* must be controlled. Dieldrin at a greater concentration is equally satisfactory. *Heliothis* species are not readily controlled by endrin or dieldrin. Of the materials which do control these pests, DDT is the most satisfactory.

DDT, endrin and dieldrin as protective sprays were effective in controlling leaf miner. Many other insecticides have limitations for use in tobacco pest control due to their short residual life, hazardous nature or tendency to cause tainting of the cured leaf.

On the basis of this work, recommendations for the control of tobacco pests in Queensland were published as the work proceeded, taking final form in an extension article by Smith (1955).

VII. ACKNOWLEDGEMENTS

Facilities for conducting the trials were provided by the Agriculture Branch of the Department of Agriculture and Stock. Dr. B. R. Champ, Mr. M. Bengston and Mr. G. W. Saunders, (Entomologists) were associated with some of the trials. The British-Australian Tobacco Company reported on samples submitted to taint tests. Mr. P. B. McGovern (Senior Biometrician in the Department) carried out all statistical analyses. All this assistance is gratefully acknowledged.

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OBSERVATIONS ON THE "BARE SOIL" METHOD OF GRASSED WATERWAY ESTABLISHMENT

By J. Rosser, Q.D.A., B.Agr.Sc.*

SUMMARY

Evidence is given that waterways on red volcanic loams possessing a subsoil resistant to erosion when undisturbed may be used to carry run-off water without prior grassing.

Where suitable plants are available, the floor of the waterway is stabilized within a few years by spread of stolons or rhizomes from plantings on the banks. Some erosion of the waterway floor may occur in the interim, but if stabilization is ensured this is of little consequence.

I. INTRODUCTION

Grassed waterways are widely used in order to provide stable conditions for the disposal of surplus run-off water. They depend on a lining of vegetation to protect them from erosion, and the establishment and maintenance of this vegetation is an important consideration in the design of schemes for soil erosion mitigation.

The waterway is usually constructed some time before it is proposed to install contour banks or other interception structures. After construction it is cultivated, fertilized and sown to grass. This grass must be given time to grow into a sward before any run-off water is allowed into the waterway. Under favourable conditions an establishment period of 12 months is usual before the waterway can be used. However, in some years, due to unfavourable seasonal conditions, it is not possible to secure a stand of grass. The occurrence of one dry growing season means that two years will go by before the contour banks can be built and the delay may be even longer. Such delays hinder soil conservation programmes and are frustrating to the farmer.

The resistance of farmers to full waterway stabilization is even more pronounced in red soil areas of Queensland, where the farmer knows by observation and experience that a waterway bare of vegetation will not necessarily turn into a deep gully. To avoid the frustration and delay, a "bare soil" method of waterway establishment has been used on the Atherton Tableland and in the Kingaroy district. This system was introduced in late 1955 and is now almost normal practice in the two districts.

In this article the "bare soil" method is described. An illustrated case history is given of one of the early bare soil waterways on the Atherton Tableland, and the results of a survey made in 1960 of 31 bare soil waterways built at Kingaroy in the seasons 1955-56 and 1956-57 are also presented.

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II. THE BARE SOIL METHOD

It should be pointed out at the outset that the method is suitable only on soil types where the subsoil in the undisturbed stage is resistant to erosion. The red volcanic loams of Atherton and Kingaroy are generally suitable and most of the writer's experience with the method has been gained on these red soils. It involves utilizing the waterway to convey run-off water immediately it is constructed. Grass is planted only on the banks, but stoloniferous or rhizomatous species are utilized. Grassing of the bed of the waterway is dependent upon the spread of grass from the banks, a process that may take several years. The final result is a grassed waterway and the run-off is carried on bare soil for only a few years.

The waterway banks are built with soil graded or dozed from the area between the banks. It is essential that only as much soil as is required for the banks be loosened by ripping or ploughing. All disturbed soil is pushed off, leaving a smooth firm bottom. Depth of excavation is dependent on the width of waterway and size of banks required. A disc or mouldboard plough is preferred to rippers or tined implements for the loosening of soil because it is difficult to excavate to the bottom of tine marks.

Waterway banks are planted on the inside batter with seed, sprigs or sod. Species that spread by stolons or rhizomes must be used, other species being unsuitable. Grasses that have been found to colonize satisfactorily are Rhodes grass (*Chloris gayana*), kikuyu grass (*Pennisetum clandestinum*) and various strains of *Cynodon dactylon,* including common couch and common African star grass.

The channel is not usually planted, because seed or runners are readily washed away, and in any case growth of grass in the undisturbed subsoil is not satisfactory. However, the plants established in the topsoil of the banks send out runners which slowly advance across the waterway. Over a period of years colonization is completed.

Probably the best results are obtained when the waterway is built through an existing stand of grass under conditions of reasonable moisture supply. In such circumstances the grass is rarely killed completely and under favourable conditions regeneration may be rapid.

III. A CASE HISTORY

The waterway chosen for this case history is one constructed on September 10, 1955, near Tolga on the Atherton Tabeland. The particular waterway is chosen because it is one of the first waterways in which the bare soil method was utilized, it carried high-velocity flows soon after construction, and photographs are available to show the progressive establishment of grass.

Soil Type.—The waterway was built on a red volcanic clay loam of basaltic origin typical of the Atherton Tableland. The original vegetation was rain-forest and the soil is known locally as a red "scrub" soil. The average land gradient at the site is 2·5 per cent.

Catchment Area.—Run-off entering the top end of the waterway is contributed by 120 ac of untreated cultivation land varying in slope from 1 to 2½ per cent. In addition, run-off from 60 ac of contour-banked cultivation with average slope of 2½ per cent. enters the waterway from contour banks, making a total area of 180 ac contributing run-off at the bottom end.

Waterway Size.—The waterway is trapezoidal in cross-section, with side slopes of 4:1. The bottom width tapers from 15 ft at the top end to 20 ft at the bottom end. Constructed bank height was 1·7 ft.

Construction Method.—The waterway was built with a large motor patrol grader. The grader's tines were used to loosen the soil. This is an undesirable practice, which resulted in this case in shallow tine marks being left in the waterway floor. These tine marks were obscured by loose soil after construction but they appeared with the first flow of water which washed the loose soil away.

Rainfall.—The average rainfall at Tolga is approximately 55 in. per annum, with a marked summer incidence. The average rainfall for each of the months December to March varies from 6 in. to 10 in., and falls of over 20 in. in any one of these months are not uncommon. Rainfall since the establishment of the waterway in 1955 has been mainly above average, as shown in Table 1.

Flow Observations.—Records of run-off are not complete but some details of flows are available for early 1956, when the waterway was still quite bare:—

On December 29, 1955, the waterway ran 1·25 ft deep.

On January 3, 1956, the waterway ran 0·75 ft deep.

On January 13, 1956, maximum surface velocity of 7 ft/sec was measured with a float. Maximum depth of flow was 0·3 ft.

On January 14, 1956, maximum surface velocity of 9 ft/sec was measured with a float. Maximum depth of flow was 0·6 ft.

On February 3, 1956, the waterway flowed 1·5 ft deep. From measurement of cross-sectional area and hydraulic radius of flow it is calculated that approximately 300 cu. ft. of water flowed down the waterway per second at an average velocity of 11 ft/sec. At this stage the waterway was still quite bare.

Table 1

TOLGA RAINFALL (INCHES), 1955–1960

—	1955	1956	1957	1958	1959	1960
January	2·89	15·12	18·99	11·91	12·49	9·74
February ..	17·45	22·48	15·72	14·88	4·98	9·48
March	25·31	15·57	5·77	13·55	16·94	2·64
April	2·80	2·05	1·58	4·71	1·88	·32
May	4·39	2·75	1·72	1·82	3·49	..
June	2·21	1·59	3·49	1·07	1·44	..
July	·70	·70	1·30	Nil	·76	..
August	·31	·59	·58	·54	1·74	..
September ..	1·09	2·29	Nil	Nil	1·71	..
October	1·56	3·88	·30	·06	Nil	..
November ..	·95	2·69	6·37	·66	4·97	..
December ..	5·36	7·90	1·23	4·35	9·66	..
Total ..	65·02	77·61	57·05	53·55	60·06	..

Although the records of flow are not complete, it is known that other flows did occur from time to time. The five flows which were recorded between December 28, 1955, and February 3, 1956, are sufficient to indicate that the waterway survived above-average flow conditions in the early stages.

Establishment of Vegetation.—The waterway was planted on January 11, 1956. A single row of sprigs was planted on each waterway bank at about original ground level. Species used were common couch (*Cynodon dactylon*) and kikuyu grass (*Pennisetum clandestinum*). Twelve months later, although these grasses were all well established, some sprigs of common African star grass (*Cynodon dactylon*) were also planted in the same position. The top and outside batter of the banks was planted with green panic (*Panicum maximum*).

Rates of Colonization.—Figures 1-4 show four stages in the grassing of this waterway. The most recent, taken in May 1959, shows the grasses meeting in places—and a bare patch 5 ft wide still to be covered in others. Had common African star grass been used from the outset, colonization would probably have been quicker. In any case, it is clear that nearly four years after establishment the waterway is still quite stable and well on the way to complete colonization by the grasses.

Fig. 1.—Waterway near Tolga on 10.ix.1955 after construction.

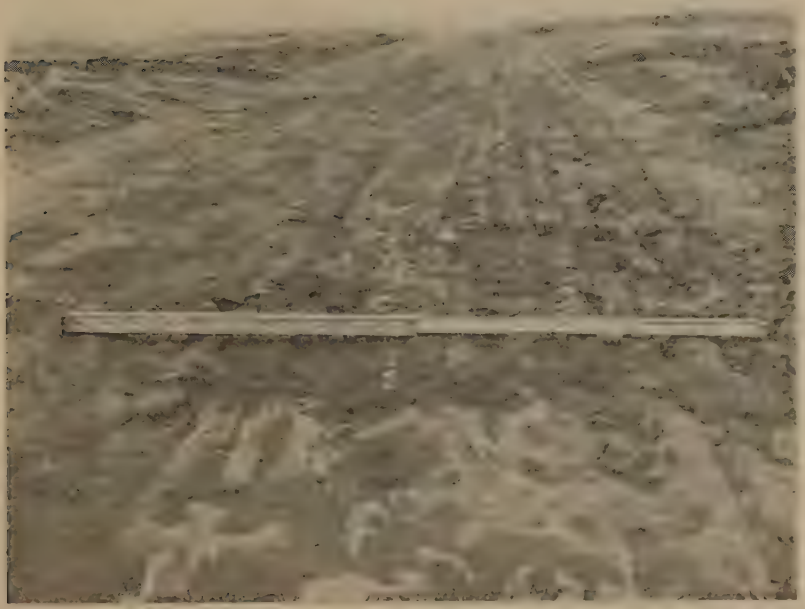

Fig. 2.—Same waterway as Fig. 1 on 16.i.1956 after four flows.

Fig. 3.—Same waterway as Fig. 1 on 17.ii.1956 after a fifth flow in which the waterway flowed at full capacity at a calculated average velocity of 11 ft./sec. The waterway floor is quite bare and perennial grasses planted on the banks have made little growth.

Fig. 4.—Same waterway as Fig. 1 on 30.v.1959. Waterway is still in good order and colonization has proceeded to the stage where grass will shortly meet in the centre.

IV. SURVEY OF 31 KINGAROY WATERWAYS

A survey of 31 waterways in the Kingaroy area was made in May 1960. The waterways had all been constructed in the period between October 1955 and November 1957, so all were at least 2½ yr old and some were 4½ yr old.

The waterways inspected represented all that could be traced from records kept at the Kingaroy office of the Department of Agriculture and Stock and are believed to represent the majority of all "bare soil" waterways constructed during this period.

Observations were made on soil type, area contributing run-off during the establishment period, waterway slope, species planted, method of planting, management, grass composition, distance of advance of grass across the waterway floor, percentage of waterway floor covered by grass, and depth of washes in waterway, if any. These aspects are listed in Tables 2 and 3 together with a record of the date of construction and a general assessment of the stability of each waterway at the date of survey. Observations were made on the last 10 chains of each waterway or on the whole waterway if it was less than 10 chains long.

Soil Types.—The waterways were located on two main soil types, red "scrub" soils and red "forest" soils. Both are red loams formed from basaltic parent material. The scrub soils are associated with rain-forest and the forest soils with sclerophyll forest. The scrub soils invariably occur on the more elevated areas of the undulating landscape and the forest soils on the lower slopes. Though both soil types have a high level of fertility, the productivity of the scrub soils is in general greater than that of the forest soils.

Considerable variation in colour, texture and apparent fertility was noted, particularly in regard to the scrub soil waterways. This may account for some of the differences both in colonization rate and in degree of erosion in the waterways.

Twenty-four of the waterways were on scrub soils and seven on forest soils.

Catchment Areas.—The catchment areas listed are those contributing at the time of establishment of the waterway. They range from 12 ac to 1,500 ac. In some cases an additional area was added at a later stage.

Waterway Slopes.—These varied from 1 to 8 per cent. Waterways with steeper gradients usually have the smaller catchments and those with small gradients have the big catchments.

Waterway Size.—Bottom widths of the waterways are recorded in Tables 2 and 3. The 24 scrub soil waterways range in bottom width from 6 ft to 38 ft, with an average of 16 ft. The seven forest soil waterways range in bottom width from 16 ft to 120 ft, with an average disturbed area of 21 ft. Three of these forest soil waterways of 100–120 ft bottom width were built with a centre strip of undisturbed grass.

Table 2

KINGAROY SCRUB SOIL WATERWAYS

Waterway No.	Date Constructed	Catchment Area (ac)	Waterway Slope (%)	Bottom Width (ft)	Species and Method of Planting	Management: Grazed between Crops	Management: Grazed Heavily	Management: Waste Area	Management: Mowed	Grass Composition: Rhodes (%)	Grass Composition: Couch (%)	Grass Composition: Paspalum (%)	Grass Composition: Kikuyu (%)	Grass Composition: African Star (%)	Percentage of Waterway Floor Grassed (%)	Distance of Advance across Waterway Floor (ft)	Depth of Gullies (ft)	Assessment of Stability
1	Aug. 1957	30	5	12	Rhodes harrowed in	*			*	95	5				100	6	0–0·2	Stable
2	Sept. 1956	35	6	10	Self-sown	*				99					95	5	0–1	Only last 100 ft gullied (should heal)
3	Sept. 1957	61	7½	10	Couch and African star hand-planted. Rhodes broadcast	*				30	70			Patch	80	2–5	·5–1·5	Couch grass established in rill ; expected to heal
4	Dec. 1955	46	5	12	Self-sown	*						99			95	6	0–2·5	Three short gullies ; may need treatment
5	Sept. 1956	53	3	10	,, ,,	*				55		45			99	5	0–1·5	Grass established in rills ; expected to heal
6	Aug. 1956	124	2½	12	Rhodes broadcast, not covered ..	*				55	45				95	6	·5–3	Rills carrying grass and partly filled with silt ; should heal
7	Aug. 1957	77	2	18	Self-sown	*				30	10		60		60	0–9	·2– ·4	Stable
8	Nov. 1957	35	3	16	Nil	*				50	50				1	0–8	0–2·5	Unstable
9	Oct. 1956	75	6	24	Rhodes broadcast ; strike poor ..	*			*	50	25		25		70	3–12	·3– ·9	Expected to heal
10	Nov. 1956	20	3	10	Self-sown	*				99					100	5	0– ·2	Stable
15	Oct. 1957	50	5	24	Rhodes broadcast	*				95	5				100	12	0– ·5	,,
17	May 1956	37	3	10	Self-sown	*				10	90				100	5	0– ·3	,,
18	Oct. 1956	19	4	16	,, ,,	*				99					100	8	none visible	,,
19	Nov. 1956	28	6	6	,, ,,	*				99					95	3	·2–1	Short rill caused by plough furrow at one place only ; stable
20	Oct. 1956	16	7	10	Spread by grader	*							99		95	5	0 2	Short rill at one place only ; now grassed up ; stable
21	Nov. 1956	200	2	24	Self-sown	*				99					50	4–12	·1– ·5	Grassing up well
22	Oct. 1955	200	2	38	,, ,,	*				80		10	10		90	14–19	0– ·5	Stable
23	Nov. 1956	110	7	20	,, ,,	*				80	20				20	0–9	·5–3	Unstable
25	Oct. 1957	45	8	16	Rhodes runners hand-planted on 10 ft grid			*		99					70	0·8	1·5–4	Unstable, but grass in bottom of gully and contour banks not affected yet
27	Nov. 1955	12	5	8	Rhodes broadcast, not covered ..	*				5		95			50	2–4	·2 1	Unstable ; needs earth weirs and couch to heal
28	Oct. 1957	12	8	8	Rhodes runners hand-planted ..		*			20	80				80	2–4	0– ·7	Stable
29	Sept. 1956	88	5	36	Rhodes broadcast, not covered ..		*			70	30				45	0–18	1–1·7	Needs weirs to heal centre rill
30	Nov. 1956	72	6	24	Rhodes broadcast	*				90	5	5			90	12	0–1	Rill caused by trickle flow after grass established
31	Nov. 1955	146	5	10	Self-sown			*		99					75	2–5	0–1	Stable ; grass growing in bottom of rill

Table 3

KINGAROY "FOREST" SOIL WATERWAYS

Waterway No.	Date Built	Catchment Area (ac)	Waterway Slope (%)	Bottom Width (ft)	Species and Method of Planting	Management: Grazed between Crops	Management: Grazed Heavily	Management: Waste Area	Management: Mowed	Grass Composition: Rhodes (%)	Grass Composition: Couch (%)	Grass Composition: Paspalum (%)	Grass Composition: Kikuyu (%)	Grass Composition: African Star (%)	Percentage of Waterway Floor Grassed (%)	Distance of Advance on to Waterway Floor (ft)	Depth of Gullies (ft)	Assessment of Stability
11	Sept./57	1500	1	120 (24)	No record	*				5	5	90			25	3–12	0– ·6	Expected to colonize and become stable
12	Nov./56	110	3	20	Rhodes broadcast and disc harrowed on banks	*				80	20				20	0–9	·1– ·8	May eventually colonize. Gaps in grass even on banks
13	Nov./56	1500	1	110 (25)	Self-sown		*				50	50			1	0–3	0– ·3	No deep gullies but grass making little headway under present management
14	Nov./56	35	2	16	,, ,,	*				99					70	3–8	0– ·2	Stable
16	Oct./57	75	3	30	Rhodes broadcast	*				99					15	0–10	·2– ·5	Erosion very slow but Rhodes grass not making much headway
24	Sept./56	42	4	16	Self-sown	*				85	15				25	0–8	·3–1·6	Unstable
26	Jan./56	650	1·3	100 (20)	,, ,,	*					10	70			99	10	0– ·5	Stable
										(plus 20% forest grasses)								

Fig. 5.—Well-managed Rhodes grass waterway at Kingaroy established by "bare soil" method. Photographed 2½ years after construction. Waterway 1 in Table 2.

Fig. 6.—Rhodes grass waterway established by "bare soil" method. Constructed in September, 1956, in a Rhodes grass paddock. Photographed 3½ years after construction. Waterway 2 in Table 2.

Fig. 7.—Scrub soil waterway stabilized by couch and Rhodes grasses. Centre rill 0.5–1.5 ft deep has common couch grass growing in the bottom. Photographed 2½ years after construction. Waterway 3 in Table 2.

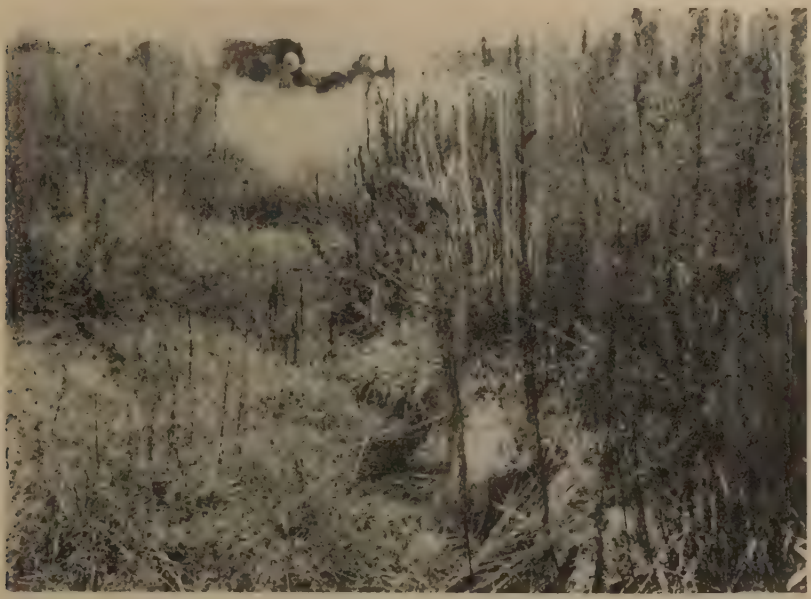

Fig 8.—This scrub soil waterway has a centre gully 0.5–3.0 ft deep. Common couch grass is stabilizing the gully, which is now partly filled with silt. Waterway 6 in Table 2.

Fig. 9.—Grass was not planted in this scrub soil waterway. Some kikuyu grass plants were accidentally established on the left bank during construction and have now spread to the centre. Waterway 7 in Table 2.

Fig. 10.—Grass was not planted in this scrub soil waterway nor has self-sown grass appeared. The centre gully is up to 2½ ft deep. Waterway 8 in Table 2.

Fig. 11.—Exposed stump roots show gradual loss of soil in a forest soil waterway. Rhodes grass is colonizing slowly in this soil type. The waterway is 2½ years old. Waterway 16 in Table 3.

Fig. 12.—A wide forest soil waterway catering for run-off from a 1,500 ac. catchment. Excavation is confined to a strip 25 ft wide on each "side." Waterway 13 in Table 3.

Fig. 13.—Of the 31 waterways inspected, this waterway showed the most serious gully development, including a gully 4 ft deep. Predominance of gravel in the profile suggests that the soil type was atypical and unsuited to the bare soil method. Waterway 25 in Table 2.

Rainfall.—Monthly rainfall for the years 1955 to 1960, together with monthly averages for a period of 54 years, are given in Table 4. It will be noted that 1955, 1956, 1958, and 1959 were years of above-average rainfall, while 1957 was a drought year.

Runoff.—No records of run-off are available. Most owners interviewed could remember waterways running strongly at some time or other in the early stages. It is reasonable to assume from rainfall figures that normal run-off occurred.

Grass Species.—Most of the waterways were found to be partly or wholly colonized by Rhodes grass (*Chloris gayana*) and/or common couch (*Cynodon dactylon*). A few carried paspalum (*Paspalum dilatatum*) and a few kikuyu grass (*Pennisetum clandestinum*). One had a section with common African star grass (*Cynodon dactylon*). A rough estimate of the percentage of each species present was made by visual observation.

Annual grasses and weeds were sometimes present on the banks but were usually in the minority on the waterway floor.

Table 4

KINGAROY RAINFALL (INCHES), 1955–1960

	1955	1956	1957	1958	1959	1960	54 Year Average 1906–1959
January ..	1·27	5·97	1·28	4·54	9·64	2·40	4·53
February ..	4·98	8·51	1·97	7·24	4·34	1·81	3·93
March	9·18	5·98	2·70	4·83	2·32	1·53	3·36
April	4·57	3·00	·21	3·17	·11	1·88	1·76
May	5·77	6·01	·20	·37	2·51	1·38	1·35
June	1·44	2·90	1·29	7·54	·19	..	1·91
July	1·55	1·28	2·98	·07	2·32	..	1·49
August	·03	·26	·97	1·97	·08	..	·96
September ..	1·28	·22	·20	1·04	3·09	..	1·49
October ..	2·86	1·90	1·79	1·84	7·99	..	2·36
November ..	1·33	1·75	·94	1·41	9·96	..	3·00
December ..	9·86	6·53	1·53	3·16	3·04	..	4·12
Total ..	44·12	44·31	16·06	37·18	45·59	..	30·26

Method of Planting.—Rhodes grass was usually planted by broadcasting seed on the banks. In most cases it was left uncovered and few good strikes were reported.

Many of the waterways were not planted at all. Where these were built through an existing stand of grass an adequate grass cover was readily established. Where they depended on spread of grass seed from adjacent areas by wind or water, results were not so satisfactory. Where paspalum was observed in waterways it could be traced to stands which grew on the waterway site. Kikuyu grass was not usually planted, but in several cases where the waterway had been constructed through a patch of this grass it was well distributed along the waterway banks, having been carried there by the graders during construction. Common couch grass is a common weed in the area and many of the waterways had it in spite of the fact that none was planted.

Management.—Few of the waterways were fenced. Most received a good spell from grazing while the adjacent area was under annual summer crops, with some grazing after the crops were harvested.

Three of the waterways appeared to be overstocked and grazed most of the time. This would be detrimental to grass colonization. A few waterways were not grazed at all at any stage. Two were mowed as required but most lacked this attention.

Extent of Colonization.—The extent of colonisation was judged by measurement of the distance of advance of the grass into the waterway floor and by an estimate of the percentage of the waterway floor covered by vegetation. As there appears to be a difference in behaviour between the scrub and the forest soils, results are discussed separately in a later section.

Distance of Advance.—Distance of advance of the grass from the waterway banks across the waterway floor was obtained by measurement. In most cases this represents growth of grass by runner from the banks but in some cases, particularly in low-gradient waterways, grass appeared to have germinated on the waterway floor.

In many cases grass had become established over the full width of the waterway. Maximum distance of advance was then taken as half the waterway width.

Percentage of Waterway Floor Covered by Vegetation.—This figure was obtained by visual observation aided by measurement of bare patches in relation to the waterway width. In the case of very wide waterways, where the grass in the centre had not been disturbed in construction, colonization figures relate to the disturbed area only.

Of the 24 scrub soil waterways, 15 had 80 per cent. or more of the bottom covered by grass; five were 100 per cent. grassed. Six more had 50–80 per cent. grassed and only three had less than 50 per cent. of the bottom covered by grass.

Of the seven forest soil waterways, one had 99 per cent. of the bottom covered by grass, one had 70 per cent. and five had less than 50 per cent. covered by grass.

Reasons can readily be found for the poor grassing of the three scrub soil waterways which were less than 50 per cent. grassed. Two had not been planted, nor had self-sown seed contributed to grass establishment; unsatisfactory grass establishment was noted on the banks. The third waterway was overgrazed.

In the case of the five forest soil waterways where less than 50 per cent. of the bottom was covered by grass, lack of suitable grass on the banks accounts for two and overgrazing for one. There is no obvious reason why grass is not better established in the other two waterways and one waterway shows clear indication that Rhodes grass is making only slow progress towards colonization (Figure 11).

All grasses appeared able to colonize the scrub soil waterways satisfactorily. Of the 21 scrub soil waterways with 50 per cent. or more of the floor colonized,

14 had Rhodes grass predominant, three common couch grass, two paspalum, and two kikuyu grass. The paspalum appeared to have germinated and established on the waterway floor rather than to have spread from the banks.

Erosion in the Waterways.—Erosion was judged by inspection and measurement of the depth of rills with line level and measuring rod. The range of depth of rills is given in Tables 2 and 3. In most cases rills were intermittent in character and in all cases gullying was confined to the centre of the waterway. In no cases had gullying reached the stage where the gully head had started to cut back into the contour banks which discharge into the waterway.

The forest soil waterways seem to have more resistance to erosion than those on scrub soils. Six out of the seven showed rilling no more than 1 ft deep and three of these had rills no more than 6 in. deep. Fifteen out of 24 scrub soils showed rilling no more than 1 ft deep, and eight of these were no more than 6 in. deep. Nine scrub soil waterways and one forest soil waterway showed rilling over 1 ft deep. Of these, five (all scrub) had rills over 2 ft deep. Maximum depth of gullying was 4 ft.

Discussion.—The 10 waterways that have developed gullies over 1 ft deep provide evidence that the method is not perfect. On the other hand, 21 waterways either have no rills or the rills are not more than 1 ft deep. Seventeen of these have more than 50 per cent. of the waterway floor grassed. Stabilization of these 17 waterways is well advanced. The four that are not grassed are forest soil waterways and the low erosion losses indicate that there is ample time available for grass establishment. It may be desirable to establish different grass species.

Of the 10 waterways with rills over 1 ft deep, five have 80 per cent. or more of the waterway floor grassed and in most cases grass is well established in the bottom of the rills. These must be conceded a good chance of stabilizing with little or no further attention.

Of the remaining five waterways which have rills over 1 ft deep and less than 80 per cent. grass, three (two scrub and one forest) were not planted and even now have large areas on the banks with no suitable grass. One is overgrazed. One has a soil profile showing a considerable amount of gravel and should not be regarded as typical red scrub soil.

V. DISCUSSION

There is sufficient evidence to demonstrate the practicability of the "bare soil" method under field conditions on red volcanic soils in Queensland. In the Atherton Tableland case reported here, erosion was limited and colonization of the waterway occurred eventually. In the Kingaroy survey, not every waterway inspected was entirely satisfactory in relation to degree of erosion and extent of

colonization, but it must be remembered that these waterways were not all given ideal treatment. For example, grass was planted in only about half of them. That a reasonable proportion was found to be completely satisfactory and that most were at least reasonably satisfactory is evidence enough that the method is practicable.

Advantages of the method are that there is no delay in the installation of contour banks and that there is no need for costly alternative waterways in lower parts of the catchment which would otherwise be necessary while grass is being established in the prepared waterway.

The advantage of being able to use the waterway immediately is based on a human failing but it is no less real and applies not only in areas where soil conservation work is in the pioneering stage. Even while they admit that the prior building and grassing of a waterway is a better method of waterway establishment and results in a superior waterway, many experienced soil conservation farmers still prefer to leave the building of the waterway until very late. They rarely plan waterway construction until they are ready to install contour banks in the paddock to be served by the waterway. The main reason is that they prefer to use the funds available in building contour banks. These provide immediate practical benefits, but a waterway is non-functional until it is used to dispose of run-off water from the contour banks.

Consideration is also given to the fact that a long delay is likely where the orthodox method of waterway establishment is adopted. Once the waterway floor is loosened to provide satisfactory conditions for seeding it cannot be used for water disposal until reasonable grass cover has been established. The time required for the establishment cannot always be predicted.

In cases where waterways are constructed in depressions already carrying run-off water, the usefulness of the "bare soil" method can be readily appreciated. No special stabilizing structures or temporary diversion banks are needed and there is no erosion outside the waterway due to run-off water that is temporarily carried there.

The main disadvantage of the method is that the waterway has little grazing value for several years. Moreover, the grass that eventually establishes on the subsoil in the waterway is rarely as vigorous or as productive as grass that is planted in a well-prepared and fertilized seedbed. On the typical Queensland cash-crop farm, where livestock production is not developed to any great extent, the waterway is not valued for its production and this disadvantage is then relatively unimportant.

A minor disadvantage is the increased cost of waterway maintenance during the initial years. Silt deposits will occur in places where velocity is reduced, e.g. where a change from high to low land gradient occurs. Removal of this silt is necessary during the establishment period.

A further disadvantage is that a rough, uneven waterway floor can develop. Small washes occur during the establishment period and although grass ultimately establishes in these rills they remain an undesirable feature, making mowing difficult and constituting a potential source of erosion in the waterway.

The successful practice of this method of waterway establishment is limited to certain soil types and to non-eroded waterway sites. Climatic conditions and availability of suitable grasses may also be limiting factors.

The basic requirement of the soil type is that it should have a firm subsoil capable of carrying water at high velocity without eroding deeply. A gradual soil loss during the establishment period is probably unavoidable and even the formation of shallow gullies over a period does not eliminate the soil type from consideration. Soils that readily form deep, sharp-sided gullies should be avoided.

Rate of erosion must be balanced against rate of colonization. Provided colonization will proceed and eventually stabilize the waterway, some erosion in the establishment period wil not matter in the long-term view.

Rate of colonization will depend on soil fertility, climate and the availability of suitable grasses. It can be expected to vary widely in different localities. The survey of Kingaroy waterways has shown that the more fertile scrub soils colonize more rapidly than do the forest soils, which are generally regarded as being less fertile.

With regard to suitable grasses, it will almost certainly be found that the grass most suitable for advancing across the waterway floor will also be equally ready to advance in the other direction, i.e. onto the cultivation. The answer to this problem lies in choosing from the species available the one that is most easily controlled by cultivation. For example, in the Kingaroy area kikuyu grass, although an excellent waterway grass in most respects, is not generally favoured by farmers because control of encroachment is difficult on the red soils. Most farmers prefer to use one of the other species mentioned earlier.

It will be obvious from the description of the method that gullied waterway sites are not suited to the "bare soil" method. Unless special precautions are taken, washing out of the gully "fill" is almost inevitable. Where possible, waterways on such sites should be grassed before use. Where this is not practicable, success can be achieved by compacting the fill, using moist soil.

VI. ACKNOWLEDGEMENT

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EFFECT OF MATURITY AND TREE AGE ON THE BEHAVIOUR OF QUEENSLAND GROWN GRANNY SMITH APPLES STORED AT 34-36°F.

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SUMMARY

The main disorder encountered during the three years of the investigation was superficial scald, which was very severe in early-picked fruit. Later picking dates significantly reduced the incidence of the disorder. The age of the tree from which the fruit was picked showed no consistent effect on the incidence of storage disorders. Picking dates earlier than mid-April resulted in heavy storage losses if the fruit was held for long periods.

I. INTRODUCTION

Investigations were initiated in 1953 into the effect of time of picking and tree age on the storage behaviour of the Granny-Smith variety of apple, grown in the Granite Belt in south-eastern Queensland and held under refrigerated conditions. The experiments were continued on a yearly basis until 1955 and were carried out in conjunction with similar experiments with the Delicious variety. Results of the Delicious investigations have already been reported by Stevenson (1959), and progress of the Granny Smith investigations was reported briefly by S. A. Trout in the Annual Reports of the Queensland Department of Agriculture and Stock for the years 1953-54 to 1955-56. More extensive reports have appeared in *Queensland Fruit and Vegetable News*, a weekly newspaper published in Queensland for local fruit and vegetable growers by the Committee of Direction of Fruit Marketing. In addition, the 1955 investigations were fully reported by Stevenson (1957) as part of a report on Apple Cool Storage Investigations carried out in that year.

II. EXPERIMENTAL DETAILS

The experimental fruit for each of the three years was obtained from the same six orchards in the Granite Belt, surrounding Stanthorpe. These orchards were selected as representative of the different growing environments within the area. Blocks of old and young trees were selcted in each orchard and all fruit used in the investigation was harvested from these trees. The age of the young trees varied between six and 13 years, and that of the old trees between 19 and 30 years. Fruit from the experimental plots was picked on three separate occasions each year. The actual picking dates are shown in Table 1.

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Table 1

PICKING DATES

—	1953	1954	1955
First Pick ..	Mar. 16–20 ..	Mar. 16–18 ..	Mar. 28
Second Pick ..	Mar. 30—Apr. 4	Mar. 30—Apr. 2	Apr. 12
Third Pick ..	Apr. 7	Apr. 12–14 ..	Apr. 27

It will be noted that the 1953 and 1954 picking dates fall into the same period, but that those in 1955 were somewhat later. This was because in the two earlier years it had been shown that there was a considerable reduction in the incidence of superficial scald with increasing maturity. In order to avoid confusion, and since the picking dates fall into four definite groups, for the sake of comparison it is proposed to refer to them in the following manner—

First Pick 1953 First Pick 1954	Mar. 16–20	Maturity 1
Second Pick 1953 Second Pick 1954 First Pick 1955	Mar. 28–Apr. 4.	Maturity 2
Third Pick 1953 Third Pick 1954 Second Pick 1955	Apr. 7	Maturity 3
Third Pick 1955	Apr. 27	Maturity 4

Two half-bushel cases from each picking date were taken from each of the blocks of old and young trees in each of six orchards in the 1953 investigations, i.e. 12 half-bushel cases from each orchard. In the two subsequent years three half-bushel cases were taken, i.e. 18 half-bushel cases from each orchard. After picking, the fruit was wrapped in oiled-paper wraps and stored at 34-36°F in the C.O.D. Cool Store, Thulimbah. Removals of fruit from store were made on two occasions in the 1953 investigations and on three occasions in the 1954 and 1955 investigations. After removal from store, the fruit was railed to Brisbane, held at air temperature for seven days to simulate normal marketing conditions, and then inspected for storage disorders.

Firmness was measured by means of a Magness Penetrometer, using the $\frac{7}{16}$ in. plunger. Five fruits from each case were taken as a sample and readings were made on opposite sides of each fruit. The means of the 10 readings were recorded.

III. RESULTS

The results are summarized in Table 3. Removal dates varied slightly from year to year but they fall into three different groupings as shown in Table 2.

Table 2

DATES OF REMOVAL OF EXPERIMENTAL FRUIT FROM STORE

—	1953	1954	1955
Removal 1 ..	Oct. 19	Oct. 4	Oct. 3
Removal 2 ..	Nov. 16	Nov. 9	Nov. 7
Removal 3 ..	..	Dec. 6	Nov. 28

In the 1953 investigations, only percentage unsound fruit was recorded and analyses of variance were made on these figures and on those for firmness. In the results from the 1954 investigations, analyses of variance were made on percentage superficial scald, percentage total breakdown and firmness, but owing to the small numbers of fruit affected by mould and soggy breakdown, an analysis was not carried out on the number of fruit affected with these disorders. Analyses of variance were made on all the disorders present in the 1955 investigations. The results are shown in Table 3.

ANALYSES OF TABLE 3

Significance of Differences in Mould, 1955

Old trees significantly less than young trees (1% level).

Significance of Differences in Soggy Breakdown, 1955

Removal 2 significantly less than Removal 3 (1% level).

Old trees significantly less than young trees (5% level).

Significance of Differences in Superficial Scald

1954

Maturity 3 significantly less than Maturity 1 (1% level).

Maturity 2 significantly less than Maturity 1 (5% level).

1955

Removal 3 significantly less than Removal 2 (5% level).

Maturity 4 significantly less than Maturity 2 and 3 (5% level).

Young trees significantly less than old trees, all removals (5% level).

Young trees significantly less than old trees, Removal 1 (5% level).

Young trees significantly less than old trees, Removal 3 (1% level).

No significant difference between young trees and old trees, Removal 2.

Table 3

ADJUSTED MEAN PERCENTAGES OF DEFECTS AND FIRMNESS OF GRANNY SMITH APPLES AFTER REMOVAL FROM COOL STORE

	Mould			Soggy Breakdown			Superficial Scald			Total Wastage			Firmness		
	1953	1954	1955	1953	1954	1955	1953	1954	1955	1953	1954	1955	1953	1954	1955
Removal 1—															
Maturity 1	*	3·3	..	*	0·3	..	*	10·4	..	68·0	14·8	..	13·1	14·8	..
Maturity 2	*	3·0	4·6	*	0·1	0·3	*	7·9	7·4	23·4	12·4	13·4	12·4	14·0	12·50
Maturity 3	*	4·3	12·5	*	Nil	0·6	*	2·6	7·9	22·4	7·3	21·8	12·1	13·2	12·25
Maturity 4	..	..	5·7	..	..	0·4	..	..	0·8	..	..	7·0	..	..	12·62
Old Trees	*	2·7	3·7	*	0·1	0·3	*	6·1	6·5	41·3	10·9	11·8	12·2	14·3	12·42
Young trees ..	*	4·3	11·2	*	0·1	0·4	*	6·9	3·4	34·6	11·7	15·2	12·8	13·7	12·50
Removal 2—															
Maturity 1	*	5·2	..	*	7·2	..	*	23·7	..	86·7	39·6	..	12·5	13·2	..
Maturity 2	*	3·7	3·7	*	6·3	8·4	*	6·9	14·4	40·0	17·4	27·2	12·1	13·2	11·88
Maturity 3	*	4·7	5·6	*	8·8	12·1	*	4·2	13·4	37·9	21·4	33·2	11·4	12·4	12·0
Maturity 4	..	..	5·8	..	..	6·3	..	..	1·0	..	..	13·2	..	..	11·83
Old Trees	*	3·6	3·4	*	6·0	8·4	*	11·3	8·5	49·4	23·8	21·8	12·3	13·1	11·94
Young trees ..	*	5·4	6·6	*	8·9	9·1	*	9·3	10·1	60·3	27·5	26·2	11·6	12·8	11·86
Removal 3—															
Maturity 1	..	3·6	..	..	18·2	..	..	22·0	..	..	50·0	..	..	12·8	..
Maturity 2	..	5·4	5·0	..	17·3	30·7	..	11·7	4·3	..	36·4	41·7	..	12·9	10·88
Maturity 3	..	8·3	5·9	..	27·0	31·0	..	5·6	5·6	..	43·2	44·2	..	12·1	10·35
Maturity 4	..	..	8·5	..	..	17·0	..	..	3·1	..	..	30·2	..	..	10·75
Old trees	..	4·6	4·5	..	7·8	18·8	..	12·2	7·2	..	38·0	33·2	..	12·8	10·46
Young trees ..	..	6·9	8·6	..	11·1	33·5	..	12·5	1·4	..	48·5	44·1	..	12·5	10·86

* Not recorded.

Significance of Differences in Total Wastage

1953

Removal 1 significantly less than Removal 2 (1% level).

Maturity 2 and 3 significantly less than Maturity 1 (1% level).

1954

Maturity 2 and 3 significantly less than Maturity 1 (1% level).

Removal 1 significantly less than Removal 2 (1% level).

Removal 2 significantly less than Removal 3 (1% level).

1955

Removal 1 significantly less than Removal 2 and 3 (1% level).

Removal 2 significantly less than Removal 3 (1% level).

Maturity 4 significantly less than Maturity 2 and 3 (1% level).

Significance of Differences in Firmness

1953

Removal 1 significantly greater than Removal 2 (1% level).

Maturity 1 significantly greater than Maturity 2 and 3 (1% level).

Old trees significantly greater than young trees (1% level).

1954

Maturity 1 and 2 significantly greater than Maturity 3 (1% level).

Removal 1 significantly greater than Removal 2 and 3 (1% level).

Old trees significantly greater than young trees (1% level).

1955

Removal 1 significantly greater than Removal 2 and 3 (1% level).

Removal 2 significantly greater than Removal 3 (1% level).

IV. DISCUSSION

(a) 1953 Results

The effect of maturity was very clearly demonstrated by the incidence of wastage. Fruit from the first pick (Maturity 1) had considerably more disorders than that from the second pick (Maturity 2) and third pick (Maturity 3). The main disorder in fruit from the first pick was superficial scald, which was reduced considerably by the use of later picking dates. Fruit from the first removal was significantly less affected than that from the second removal. The age of the trees had no effect on the amount of wastage present.

Firmness was significantly affected by the maturity of the fruit, the age of the trees from which it was picked, and the length of time it was held in store. Fruit from the first removal was significantly firmer than that from the second removal, while that from the first pick was significantly firmer than that from the two subsequent picks. In addition, fruit from old trees was significantly firmer than that from young trees.

(b) 1954 Results

Only a small number of fruit was affected by mould and an analysis of variance was not made on the results. There was, however, a slight increase in the incidence of mould with increase in the length of the storage period. The maturity of the fruit did not affect the susceptibility to mould in the first and second picks, but the results indicate that fruit from the third pick was slightly more susceptible, particularly in fruit from the third removal. Fruit from old trees was less affected than that from young trees.

There was a considerable increase in the incidence of soggy breakdown with increase in the storage period. The date of picking did not greatly affect the amount of the disorder present. However, the results indicate that fruit from the third maturity was more susceptible to the disorder than that from earlier maturities, particularly as the storage period increased. Fruit from old trees was less affected than that from young trees.

The effect of maturity on superficial scald was very marked. Fruit from the second and third picks had significantly less scald than that from the first pick. This effect of decrease in scald incidence with increase in maturity has been reported by Padfield (1950) in Granny Smith apples, and by Stevenson (1959) in Delicious apples. Tindale and Huelin (1939) claimed that the reduction in scald occurs only over a short period, after which there is a marked increase in the disorder with further increase in maturity. An increase in the length of the storage period from six months to nine months did not affect the incidence of scald. Fruit from young and old trees showed no variation in its susceptibility to the disorder.

An analysis of variance made on total wastage, which comprised mould, soggy breakdown and superficial scald, clearly indicated the effect of maturity. Fruit from the first pick had significantly more wastage than that from the other two picks. The amount of wastage significantly increased as the length of the storage period increased. Significant differences were shown to exist between fruit from young and old trees.

The firmness of the fruit was affected by maturity, tree age and the length of the storage period. Fruit from the first and second pick was significantly firmer than that from the third pick. In addition, fruit from the first removal was significantly firmer than that from the second and third removals. Fruit from old trees was significantly firmer than that from young trees.

(c) 1955 Results

Only a small number of fruit was affected by mould, but fruit from young trees was more susceptible than fruit from old trees. There was no increase in mould incidence with increase in the length of the storage period, nor was the susceptibility of the fruit affected by the time of picking.

Soggy breakdown was found to be significantly increased with increase in the length of the storage period but its incidence was not affected by the time of picking. Fruit from old trees was less susceptible than that from young trees.

The effect of time of picking on the incidence of superficial scald was most noticeable. Wastage due to this disorder decreased considerably with later picking dates. It should be noted that the picking dates used in the 1955 investigations were later than those used in previous years. In the 1954 investigations tree age had no effect on the incidence of superficial scald but in 1955 fruit from young trees had less scald than that from old trees in two of the three removals. Fruit from the third pick had less total wastage than that from either of the other two picks, and increase in storage time increased the total wastage. A significant difference due to age of trees was recorded.

The only factor that affected the firmness of the fruit was the length of the storage period. Fruit became softer as the length of the storage period increased.

V. CONCLUSIONS

The results of the three years' investigations clearly indicate the effect of picking maturity. Fruit picked in March was particularly susceptible to superficial scald, but by picking the fruit in mid-April the incidence of this disorder was considerably reduced. Since the major disorder was superficial scald, the amount of total wastage was thus considerably reduced. Picking dates later than April 27 have yet to be studied to determine how far this effect of maturity on scald incidence continues.

Tree age had no effect on the incidence of disorders except in the 1954 experiments, when fruit from young trees had significantly less superficial scald than that from old trees.

From the results obtained it appears that the Granny Smith variety from the Granite Belt for long storage should not be picked before mid-April. The question as to whether better results could be obtained by picking the fruit later than mid-April has yet to be determined. However, in later pickings frost injury may occur. In order to keep storage disorders to a minimum the fruit should not be held after mid-October at the temperatures studied.

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STUDIES ON THE CAUSE OF A LOW COPPER STATUS IN CATTLE IN SOUTH-EASTERN QUEENSLAND

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SUMMARY

Four experiments were made to compare the copper/molybdenum/inorganic sulphate relationship in the diets of sheep and cattle and to determine if this relationship was responsible for the low copper status in cattle grazing pastures at the Animal Husbandry Research Farm at Rocklea, near Brisbane.

Experiment 1 measured changes in liver copper concentrations in four groups of two sheep on (1) a basal diet of oaten and lucerne chaff, (2) this diet supplemented with molybdenum, (3) this diet supplemented with copper plus molybdenum, and (4) this diet supplemented with copper plus molybdenum plus inorganic sulphate. The findings confirmed that sheep can increase their liver copper reserves on a ration containing 2.5 p.p.m. Cu, that a high concentration of molybdenum (23 p.p.m. Mo) does not interfere with copper metabolism when the diet is low in inorganic sulphate (0.02 per cent.) and crude protein (4.4 per cent.) and that the addition of inorganic sulphate to provide 0.45 per cent. SO_4 permits the molybdenum to interfere with copper metabolism.

Experiment 2 was similar in design to Experiment 1 but included a comparison of sheep and cattle. The basal diet contained 9 per cent. linseed meal to give a productive ration of 8.2 per cent. crude protein. Findings were similar for sheep and cattle. Both species showed little decline in liver copper on a diet containing 4 p.p.m. Cu, 0.25 p.p.m. Mo and 0.02 per cent. SO_4. The addition of molybdenum to provide 11.5 p.p.m. Mo in this protein supplemented diet caused a marked fall in liver copper although the diet was low in inorganic sulphate. The further addition of inorganic sulphate as sodium sulphate to give 0.45 per cent. SO_4 caused an increased interference with copper metabolism.

Experiment 3 included a stall feeding treatment to examine the copper/molybdenum/sulphate relationship at the levels found in freshly cut pasture, a comparison of the changes in liver copper levels in cattle fed freshly cut pasture in stalls and in cattle grazing similar pasture, and a long-term experiment to determine changes in liver copper concentrations in cattle grazing these pastures over one year. Essential findings were (1) grazing cattle showed a marked fall in liver copper reserves throughout the year, (2) this rate of decline in liver copper levels in grazing cattle was greater than in cattle stall-fed on freshly cut pasture and (3) inability to metabolize copper did not appear to be related either to the copper content or to the molybdenum level in these pastures.

Experiment 4 was firstly a measure of the rate of decline in liver copper levels in cattle grazing these pastures for 12 weeks up to the time at which the pasture was conserved as hay. The second stage was a stall-feeding experiment to examine the copper/molybdenum/sulphate relationship in a diet of pasture hay. Essential findings were (1) grazing cattle, as

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in Experiment 3, showed a marked fall in liver copper levels, (2) the decline in liver copper levels was greatest in animals during the grazing period, less in animals fed paspalum hay and least in animals fed a basal diet with a copper, molybdenum, inorganic sulphate and crude protein content similar to that of the paspalum hay and (3) as in Experiment 3 inability to metabolize copper could not be related either to the copper content or to the molybdenum level in these pastures.

From the findings of all four experiments the conclusion is drawn that the predominantly paspalum pasture at the Animal Husbandry Research Farm, Rocklea, contains some factor or factors, other than molybdenum in the presence of inorganic sulphate, which interfere with metabolism of copper by cattle.

I. INTRODUCTION

It has been known for some years that copper deficiency, as indicated by low liver copper reserves and/or low blood copper concentrations, is widespread in both sheep and cattle in Queensland (Harvey 1952). The occurrence and correction of copper deficiency in sheep in north-western Queensland were described by Moule, Sutherland, and Harvey (1959). These workers stated that copper deficiency in sheep in this locality was due partly to the low copper and partly to the high molybdenum and inorganic sulphate levels in the predominant vegetation.

Copper deficiency in cattle in south-eastern Queensland was described by Sutherland (1952). Alexander and Harvey (1957) showed that a low copper status in cattle occurred on 95 of 127 farms situated on the alluvial areas in south-eastern Queensland. Sutherland (1956) reported that cattle grazing the predominantly *Paspalum dilatatum* pasture at the Animal Husbandry Research Farm at Rocklea, Brisbane, showed very low liver copper levels. He stated also that since the copper content of the dominant pasture species was adequate, it was apparent that there must be some interference with the metabolism of copper by cattle.

Cunningham (1950) confirmed and amplified the findings of Dick and Bull (1945) that a high molybdenum intake could reduce liver copper storage in both sheep and cattle. Dick (1952, 1953) established that molybdenum in the presence of inorganic sulphate interferes with copper metabolism in sheep. However, Allcroft and Lewis (1956) reported that molybdenum and inorganic sulphate were not the only factors which limit copper storage in sheep and cattle on diets of apparently adequate copper content. Mylrea (1958) showed that the addition of sulphate to diets low in sulphate (0·03 per cent.) and containing 2·4 p.p.m. molybdenum and 7·6 p.p.m. copper caused a decline in liver copper levels in cattle. Cunningham, Hogan, and Lawson (1959) stated that cattle are more sensitive than sheep to high molybdenum levels in pasture. They suggested that the greater sensitivity of cattle is related to the low level of accompanying inorganic sulphate that is necessary to cause loss of copper.

The investigations reported in this paper were undertaken to examine the cause of the low copper status in cattle grazing the predominantly *Paspalum dilatatum* pastures at the Animal Husbandry Research Farm, Rocklea. Four experiments are reported. Experiment 1 was designed to examine the copper/molybdenum/sulphate relationship in sheep on a diet of oaten chaff plus 5 per cent. lucerne chaff. Experiment 2 was to compare the copper/molybdenum/sulphate relationship for sheep and cattle on a diet of oaten chaff, lucerne chaff and linseed meal. Experiment 3 was to examine the copper/molybdenum/sulphate relationship at the levels found in the predominantly *Paspalum dilatatum* pasture. This pasture, which was cut and fed daily, showed considerable variation in chemical composition during the experimental period. Experiment 4 was designed to evaluate the copper/molybdenum/sulphate relationship at the levels encountered in hay made from this pasture in early summer.

II. METHODS AND MATERIALS

(a) Experimental Animals

The sheep in Experiment 1 were selected from a uniform line of Merino wethers, approximately 14 months of age and obtained three months previously from the Roma district in southern Queensland. The sheep in Experiment 2 were from a uniform line of full-mouth Merino wethers which had been in the experimental flock for three years.

All cattle used in these experiments were Hereford steers sired by the same bull and born and reared on the pastures of the Animal Husbandry Research Farm, Rocklea. In Experiment 2, weaners aged 6–7 months were used. In Experiment 3 the experimental animals were 11 steers aged approximately 12 months and four steers aged 24 months. The former included the eight steers used previously in Experiment 2. The nine steers used in Experiment 4 were approximately 12 months of age.

As all cattle grazing the pastures on the Animal Husbandry Research Farm show low copper levels in liver, copper therapy by intravenous injection of copper sulphate was given approximately three weeks prior to the commencement of each experiment.

Liver biopsies on sheep were made by the techniques of Dick (1944). Liver samples from cattle were obtained by the biopsy technique of Loosmore and Allcroft (1951). The instruments described by Dick (1952) were used for biopsies in both sheep and cattle.

Blood samples were drawn from the jugular vein, using stainless steel needles; the blood was collected in copper-free bottles containing potassium oxalate.

(b) Experimental Facilities

The sheep were housed in wooden metabolism crates, and the cattle in individual stalls provided with concrete floors and individual concrete feeding troughs.

(c) Body-weight

Body-weight of sheep in Experiments 1 and 2 was measured with a clock-face spring balance with an accuracy of ± 0·5 lb. Cattle were weighed on a cattle weighing scale with an accuracy of ± 2 lb. Both sheep and cattle were weighed on all occasions at a standard time before the morning feed and prior to obtaining liver samples.

(d) Chemical Methods

Methods used in analyses of feeds were essentially those of the Association of Official Agricultural Chemists (1955). Copper in feed, blood and liver was determined by the method of Clare, Cunningham, and Perrin (1945). The method of Dick and Bingley (1951) was used for the determination of molybdenum. Inorganic sulphate was determined by the modification of the benzidene sulphate method of J. B. Bingley and A. T. Dick (personal communication 1953).

(e) Experimental Rations

A quantity of oaten chaff, lucerne chaff, linseed meal and pulverized limestone sufficient for all experiments was purchased prior to the commencement of these studies. The oaten chaff was a uniform line of poor quality containing virtually no grain. The lucerne chaff was of fair quality. Both were individual consignments from a property.

Rations were compounded in a rotary feed mixer. Additional copper, molybdenum and inorganic sulphate were in the form of chemically pure copper sulphate, ammonium molybdate and sodium sulphate respectively. The appropriate chemical was dissolved in water and the solution sprayed as a fine mist on the ration as it rotated in the feed mixer. Analyses of samples confirmed the adequacy of mixing.

The paspalum used in Experiment 3 was harvested, chaffed and fed daily. This material was cut from the paddock in which the experimental grazing group was maintained.

The paspalum hay used in Experiment 4 was prepared from the paddock in which all animals in this experiment were maintained prior to the stall-feeding portion of this study.

Representative samples for chemical analyses were collected at intervals during the course of these experiments. In Experiment 3 the dry-matter content

was determined daily on the freshly cut paspalum; these dried samples were bulked and analysed at weekly intervals; all feed residues were sampled and determined as dry matter.

The proximate analyses on a dry-matter basis are shown in Table 1 for individual feeds, compounded rations, paspalum pasture and paspalum hay used in Experiments 1–4. The levels recorded for paspalum and rations in Experiment 3 are average values from weekly analyses; figures in parentheses show the range of crude protein and copper levels.

III. EXPERIMENT 1

(a) Experimental

The eight sheep were distributed at random to four groups of two sheep, and the groups were allotted at random to the treatments as follows:—

Group I: Basal ration of 94 per cent. oaten chaff, 5 per cent. lucerne and 1 per cent. limestone.

Group II: Basal ration + ammonium molybdate to increase the molybdenum content to 23 p.p.m. Mo.

Group III: Basal ration + ammonium molybdate + copper sulphate to provide 23 p.p.m. Mo and 21 p.p.m. Cu.

Group IV: Basal ration + ammonium molybdate + copper sulphate + sodium sulphate to give 23 p.p.m. Mo, 21 p.p.m. Cu and 0·45 per cent. SO_4.

Sheep were fed *ad lib.* for the 10 weeks of this experiment. Individual daily feed consumption was determined.

Liver biopsy samples for copper analyses were taken and body-weight was recorded at the commencement and at the conclusion of this experiment.

Blood samples for the determination of copper and molybdenum were drawn initially, after four weeks and at the conclusion of the 10 weeks of this experiment.

(b) Results

The concentration in the diet and the average daily intake of copper, molybdenum and inorganic sulphate of sheep are shown in Table 2.

Table 1

COMPOSITION ON DRY-MATTER OF INDIVIDUAL FEEDS, COMPOUNDED RATIONS, PASTURE AND HAY FED IN EXPERIMENTS 1 TO 4

—	Protein (%)	Fat (%)	Fibre (%)	NFE (%)	Ash (%)	Calcium (% Ca)	Phosphorus (% P)	Sulphate (% SO_4)	Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Manganese (p.p.m. Mn)
Individual Feeds—											
Oaten chaff ..	3·7	2·7	30·3	56·8	6·5	0·10	0·11	0·01	2·5	0·1	25·7
Lucerne chaff ..	17·8	1·5	30·8	41·4	8·5	0·89	0·36	0·07	6·0	0·9	33·7
Linseed meal ..	40·2	3·9	9·0	40·8	6·1	0·23	1·07	0·22	17·4	0·7	38·1
Limestone ..						39·2		0·01	2·0	0·4	17·5
Experiment 1—											
Basal ration ..	4·4	2·6	30·0	55·6	7·4	0·47	0·12	0·02	2·5	0·2	25·8
Experiment 2—											
Basal ration ..	8·2	2·7	28·3	53·7	7·1	0·56	0·22	0·02	4·0	0·2	26·9
Experiment 3—											
Basal ration (Av.)	5·5 (5·0–6·4)*	2·6	30·0	55·2	6·7	0·21	0·14	0·02	5·8 (5·0–6·5)*	0·2	26·9
Paspalum (Av.) ..	5·2 (4·2–7·0)*	1·6	37·3	48·2	7·7	0·25	0·16	0·35	5·9 (5·0–6·5)*	0·2	25·0
Experiment 4—											
Basal ration ..	7·1	2·7	29·1	54·4	6·7	0·17	0·18	0·02	6·5	0·2	28·0
Paspalum hay ..	7·2	1·2	37·6	45·4	8·6	0·15	0·22	0·35	6·5	0·1	27·4

* Figures in parentheses show the range in values obtained from weekly analyses.

Table 2

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF SHEEP IN EXPERIMENT 1

Group	Sheep No.	Concentration in Diet			Mean Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	i	2·5	0·2	0·02	0·8	<0·01	0·07
	ii	2·5	0·2	0·02	0·7	<0·01	0·06
II	iii	2·5	23·0	0·02	0·6	5·0	0·04
	iv	2·5	23·0	0·02	0·9	8·2	0·07
III	v	21·0	23·0	0·02	7·1	7·8	0·07
	vi	21·0	23·0	0·02	6·7	7·4	0·06
IV	vii	21·0	23·0	0·45	8·0	8·7	1·71
	viii	21·0	23·0	0·45	5·9	6·4	1·26

Despite the inclusion of 5 per cent. lucerne chaff and 1 per cent. limestone in the basal ration, feed intake was most unsatisfactory and it was not possible to maintain the planned daily intake of 10 mg copper and 10 mg molybdenum even though the high concentrations of 21 p.p.m. Cu and 23 p.p.m. Mo were used in the supplemented rations. Because of the unsatisfactory feed intake the experiment was terminated after 10 weeks.

Changes in body-weight and in liver copper concentrations of the individual sheep are shown in Table 3. In spite of the low copper content in the basal ration, the sheep in Group I tended to at least maintain liver copper levels. This is emphasised in Group II where although additional molybdenum was added to the basal ration the sulphate level was sufficiently low for the molybdenum to have no influence and sheep increased liver copper levels.

Table 3

BODY-WEIGHT AND COPPER CONCENTRATIONS IN LIVER OF SHEEP IN EXPERIMENT 1

Group	Sheep No.	Body-weight (lb)			Copper Concentration (p.p.m. Cu)			
		Initial	After 10 Weeks	Mean Group Change	Initial	After 10 Weeks	Change	Mean Group Change
I	i	46	42	−4	428	373	− 55	+ 53
	ii	44	40		228	389	+161	
II	iii	42	35	−5	429	496	+ 67	+ 73
	iv	46	44		281	359	+ 78	
III	v	46	43	−4	297	611	+314	+313
	vi	42	38		224	536	+312	
IV	vii	44	45	−4	487	541	+ 54	+ 60
	viii	50	43		306	372	+ 66	

In Group III the increased copper intake caused a marked increase in liver copper levels in spite of the high molybdenum content of the diet. As in Group II the inorganic sulphate level was low—0·02 per cent.

In Group IV the addition of sulphate to a ration similar to that of Group III prevented the increase in liver copper shown by sheep in Group III.

The copper and molybdenum levels in blood are shown in Table 4. The blood copper levels were similar in all groups and would be considered normal. The blood molybdenum levels were high in sheep from Groups II and III, where the diet was high in molybdenum and low in sulphate. These high levels were not attained in sheep from Group IV, where additional sulphate was added to the high molybdenum diet.

Table 4

COPPER (mg Cu/100 ml) AND MOLYBDENUM (μg Mo/100 ml) IN BLOOD OF SHEEP IN EXPERIMENT 1

Group	Sheep No.	Initial		After 4 Weeks		After 10 Weeks	
		Cu	Mo	Cu	Mo	Cu	Mo
I	i	0·09	2·0	0·12	2	0·12	2
	ii	0·11	2·0	0·12	2	0·10	11
II	iii	0·12	2·0	0·10	360	0·09	735
	iv	0·09	2·0	0·12	875	0·10	1300
III	v	0·11	2·0	0·12	1025	0·12	1675
	vi	0·12	2·0	0·12	1025	0·12	1085
IV	vii	0·13	2·0	0·14	17	0·12	30
	viii	0·10	2·0	0·13	27	0·12	30

IV. EXPERIMENT 2

(a) Experimental

The eight sheep and eight cattle were distributed at random to four groups each containing two sheep and two cattle. The groups were allotted at random to the treatments as follows:—

Group I: Basal ration of 81 per cent. oaten chaff, 9 per cent. lucerne, 9 per cent. linseed meal and 1 per cent. limestone.

Group II: Basal ration + ammonium molybdate to increase the molybdenum concentration to 11·5 p.p.m. Mo.

Group III: Basal ration + ammonium molybdate + copper sulphate to provide 11·5 p.p.m. Mo and 13 p.p.m. Cu.

Group IV: Basal ration + ammonium molybdate + copper sulphate + sodium sulphate to provide 11·5 p.p.m. Mo, 13 p.p.m. Cu and 0·45 per cent. SO_4.

Both sheep and cattle were fed slightly below appetite to minimize feed residues and thus selectivity. An attempt was made to maintain comparable feed intake for all sheep and for all cattle. Individual daily feed consumption was determined.

Body-weight was recorded initially and again at the conclusion of the 12 weeks of this experiment.

Liver samples for copper analysis were obtained at the commencement and at the conclusion of the experiment. An additional sample from cattle was taken when the animals had been on the experimental ration for six weeks.

Blood samples for the determination of copper and molybdenum were drawn initially, after four weeks, after eight weeks and at the conclusion of the 12 weeks of this experiment.

(b) Results

The concentration in the diet and the average daily intake of copper, molybdenum and inorganic sulphate of sheep and cattle are shown in Table 5. The mean feed intake of sheep was 800 g daily except for the lighter animal in Group III, which consumed an average of 700 g per day. The mean daily feed intake of cattle ranged from 4·01 kg to 4·08 kg.

Table 5

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF SHEEP AND CATTLE IN EXPERIMENT 2

Group	Animal No.	Concentration in Diet			Mean Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	Sheep 1	4·0	0·25	0·02	3·2	0·2	0·16
	Sheep 2				3·2	0·2	0·16
	Cattle 1				16·1	1·0	0·80
	Cattle 2				16·2	1·0	0·81
II	Sheep 3	4·0	11·5	0·02	3·2	9·2	0·16
	Sheep 4				3·2	9·2	0·16
	Cattle 3				16·1	46·3	0·80
	Cattle 4				16·3	46·9	0·82
III	Sheep 5	13·0	11·5	0·02	10·4	9·2	0·16
	Sheep 6				9·1	8·1	0·14
	Cattle 5				53·1	47·0	0·82
	Cattle 6				52·9	46·8	0·81
IV	Sheep 7	13·0	11·5	0·45	10·4	9·2	3·6
	Sheep 8				10·4	9·2	3·6
	Cattle 7				52·1	46·1	18·0
	Cattle 8				53·1	46·9	18·4

As the protein level in the diet and the feed consumption had been unsatisfactory in Experiment 1 it was considered desirable to increase the lucerne chaff content from 5 per cent. to 9 per cent. and to include 9 per cent. linseed meal. This gave a palatable, productive ration. The increased molybdenum concentration (11·5 p.p.m. Mo) in rations for Groups II, III and IV and the increased copper

levels (13·0 p.p.m. Cu) in rations for Groups III and IV were to maintain a mean daily intake of approximately 10 mg molybdenum and 10 mg copper in sheep.

Changes in body-weight and in liver copper concentration of individual animals are shown in Table 6. In all groups the mature sheep used in this experiment maintained body-weight, whereas the weaner steers gained weight at the rate of approximately 1 lb per head per day.

Table 6

BODY-WEIGHT AND COPPER CONCENTRATION IN LIVER OF SHEEP AND CATTLE IN EXPERIMENT 2

Group	Animal No.	Body-weight (lb)			Copper Concentration (p.p.m. Cu)					
		Initial	After 12 Weeks	Change	Initial	After 6 Weeks	After 12 Weeks	Total	Change Group Mean	
									Sheep	Cattle
I	Sheep 1	88	88	0	453		452	− 1	− 5	
	Sheep 2	88	85	− 3	367		359	− 8		
	Cattle 1	367	479	+112	112	105	105	− 7		−14
	Cattle 2	371	462	+ 91	123	118	102	− 21		
II	Sheep 3	83	81	− 2	373		211	−162	−129	
	Sheep 4	82	86	+ 4	414		320	− 94		
	Cattle 3	427	510	+ 83	124	68	40	− 84		−94
	Cattle 4	357	452	+ 95	155	87	52	−103		
III	Sheep 5	80	81	+ 1	492		537	+ 45	+ 80	
	Sheep 6	72	73	+ 1	473		587	+114		
	Cattle 5	369	474	+105	137	166	189	+ 52		+49
	Cattle 6	396	471	+ 75	119	133	165	+ 46		
IV	Sheep 7	85	85	0	360		210	−150	− 99	
	Sheep 8	88	88	0	412		365	− 47		
	Cattle 7	414	500	+ 86	136	108	91	− 45		−54
	Cattle 8	337	447	+110	147	104	85	− 62		

In spite of the low copper content of the basal ration (4 p.p.m. Cu), both sheep and cattle in Group I showed little change in liver copper reserves during the 12 weeks of this experiment.

In Group II the addition of molybdenum caused a marked decrease in liver copper reserves in both sheep and cattle. This occurred in spite of the low level of inorganic sulphate. These findings are contrary to those of Group II in Experiment 1, where a marked increase occurred in liver copper reserves on a diet equally low in sulphate, even lower in copper and containing approximately twice the concentration of molybdenum. An essential difference between the two experiments was the twofold increase in the crude protein content of the basal ration in Experiment 2.

In Group III both sheep and cattle showed an increase in the liver copper reserves due to the higher copper level in the diet. The magnitude of this increase was much lower than for Group III in Experiment 1.

In Group IV both sheep and cattle showed a marked fall in liver copper levels due to the additional sulphate in the rations fed to this group.

The copper and molybdenum levels in blood are shown in Table 7. The blood copper levels tended to be higher in sheep than in cattle. As in Experiment 1, the blood molybdenum levels were high in animals from Groups II and III, where the diet was high in molybdenum and low in sulphate. Similarly, these high levels were not attained in animals in Group IV on the same high molybdenum diet but with additional sulphate.

Table 7

COPPER (mg Cu/100 ml) AND MOLYBDENUM (μg Mo/100 ml) IN BLOOD OF SHEEP AND CATTLE IN EXPERIMENT 2

Group	Animal No.	Initial		After 4 Weeks		After 8 Weeks		After 12 Weeks	
		Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo
I	Sheep 1	0·12	<2	0·11	2	0·10	2	0·09	6
	Sheep 2	0·12	<2	0·10	2	0·09	4	0·09	6
	Cattle 1	0·08	<2	0·08	<2	0·06	<2	0·06	<2
	Cattle 2	0·08	<2	0·06	<2	0·06	<2	0·06	<2
II	Sheep 3	0·10	<2	0·09	654	0·10	756	0·10	698
	Sheep 4	0·10	<2	0·10	716	0·09	756	0·08	708
	Cattle 3	0·09	<2	0·06	160	0·06	40	0·06	47
	Cattle 4	0·09	<2	0·08	164	0·06	49	0·06	53
III	Sheep 5	0·08	<2	0·09	522	0·07	837	0·08	961
	Sheep 6	0·09	<2	0·13	383	0·10	656	0·09	749
	Cattle 5	0·08	<2	0·08	137	0·06	91	0·06	71
	Cattle 6	0·08	<2	0·08	119	0·06	36	0·06	62
IV	Sheep 7	0·09	<2	0·12	12	0·10	12	0·08	8
	Sheep 8	0·09	<2	0·11	16	0·07	8	0·07	10
	Cattle 7	0·08	<2	0·08	8	0·06	4	0·06	10
	Cattle 8	0·09	<2	0·08	7	0·10	2	0·06	8

V. EXPERIMENT 3

(a) Experimental

The 15 Hereford steers were distributed to give five groups of three animals. For Groups I, II and III, nine steers aged approximately 12 months were allocated on a body-weight basis. Groups IV and V each contained two steers aged approximately 24 months and one steer aged about 12 months and allocation within these two groups was on a body-weight basis.

Group I: Basal ration of 90 per cent. oaten chaff and 10 per cent. lucerne chaff, plus sufficient linseed meal and copper sulphate to give the same protein and copper levels as those found in the

paspalum pasture mown and fed to Groups III and IV. As this pasture changed in chemical composition with stage of growth, samples were taken daily, oven-dried and bulked to give a weekly composite sample. This was analysed and the necessary adjustments were made at weekly intervals to the amount of linseed meal and copper sulphate added to the basal ration.

Group II: As for Group I, plus sodium sulphate to provide a sulphate level equal to that in paspalum fed to Groups III and IV. As in Group I, adjustments were made at weekly intervals to simulate in this ration the protein, copper, molybdenum and sulphate levels found in the cut paspalum pasture. Additional ammonium molybdate was not required as the molybdenum content of the basal ration was comparable with that of paspalum.

Group III: Paspalum pasture cut and fed daily. The amount fed was slightly below appetite and was adjusted so that all animals in Groups I, II and III had approximately the same daily dry-matter intake.

Group IV: Paspalum pasture cut daily and fed *ad lib.*

Group V: Grazing the same pasture from which the paspalum fed to Groups III and IV was taken.

Individual daily dry-matter feed consumption was measured for each of the animals in Groups I-IV.

Body-weight was determined initially and at the conclusion of the 12 weeks of the experiment.

Liver biopsy samples for copper analyses were taken initially, after six weeks and at 12 weeks when the experiment was concluded.

At the conclusion of the stall-feeding experiment the three animals from Group IV were transferred to grazing on Rocklea pastures. Liver samples were taken for analysis for copper at approximately monthly intervals. Whenever liver copper concentrations fell below 50 p.p.m. copper therapy by intravenous injection of copper sulphate was given to raise the liver copper reserves to about 150 p.p.m. Cu. The observation continued for 12 months. Body-weight was recorded at approximately monthly intervals.

(b) Results

The concentration in the diet and the average daily intake of copper, molybdenum and inorganic sulphate of the experimental cattle are shown in Table 8. These are average figures, as the chemical composition varied with changes in the stage of growth during the experimental period from February to May.

Table 8

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF CATTLE IN EXPERIMENT 3

Group	Tag	Concentration in Diet			Average Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	A	5·8	0·2	0·02	23·8	0·8	0·82
	B				24·4	0·8	0·84
	C				24·4	0·8	0·84
II	D	5·8	0·2	0·35	24·4	0·8	14·7
	E				24·4	0·8	14·7
	F				24·4	0·8	14·7
III	G	5·9	0·2	0·35	23·5	0·8	14·0
	H				23·5	0·8	14·0
	I				24·3	0·8	14·4
IV	J	5·9	0·2	0·35	32·9	1·1	19·5
	K				32·9	1·1	19·5
	L				29·9	1·0	17·8
V	M	} Cattle grazing pasture from which diets of Groups III and IV were taken					
	N						
	O						

Changes in body-weight and in liver copper concentrations are shown in Table 9. Cattle tended to maintain or gain weight in Groups I, II and V and to lose weight in Groups III and IV. These findings suggest that the basal ration was of higher nutritive value than the paspalum. They also indicate that the grazing animals in Group V were able to select a better quality diet than the pasture cut and fed *ad lib.* to comparable animals in Group IV.

Table 9

COPPER CONCENTRATIONS IN LIVER AND BODY-WEIGHT OF CATTLE IN EXPERIMENT 3

Group	Tag	Body-weight (lb)				Copper Concentration (p.p.m. Cu)				
		Initial	After 12 Weeks	Change		Initial	After 6 Weeks	After 12 Weeks	Change	
				Total	Group Mean				Total	Group Mean
I	A	620	632	+12		133	128	94	−39	
	B	560	552	− 8	+ 7	114	95	107	− 7	−22
	C	492	508	+16		135	107	114	−21	
II	D	520	564	+44		199	129	109	−90	
	E	548	580	+32	+20	170	128	115	−55	−63
	F	708	692	−16		201	175	158	−43	
III	G	552	512	−40		176	139	184	+ 8	
	H	572	556	−16	−25	221	177	173	−48	−16
	I	524	504	−20		189	160	182	− 7	
IV	J	852	796	−56		175	128	135	−40	
	K	816	788	−28	−27	162	179	177	+15	+ 8
	L	720	724	+ 4		205	193	253	+48	
V	M	816	808	− 8		154	118	107	−47	
	N	856	912	+56	+32	158	117	105	−53	−57
	O	732	780	+48		163	119	92	−71	

The mean changes in liver copper reserves showed a slight fall in Groups I and III, a marked fall in Groups II and V and a small gain in Group IV. Thus there is an indication that the addition of sodium sulphate to the diet of Group II adversely affected the availability of the dietary copper. This finding was not confirmed subsequently in Experiment 4. There is also an indication that the moderately low copper (5.9 p.p.m.) in freshly cut paspalum pasture at this season (February to May) is sufficient to permit stalled cattle on *ad lib.* feeding to remain in copper balance. A comparison of data from Groups IV and V suggests that grazing cattle select a diet which is higher in total digestible nutrients but lower in "available" copper.

The mean changes in body-weight and in liver copper concentration of cattle grazing Rocklea pastures from June 21, 1957, to June 20, 1958, are shown in Figure 1. Cattle gained in body-weight from August to May. All animals showed a marked fall in liver copper throughout the year, the rate of decline tending to decrease as the copper concentration in liver became less.

Fig. 1.—Mean changes in body-weight and liver copper concentrations in grazing cattle.

VI. EXPERIMENT 4

(a) Experimental

Nine Hereford steers approximately 12 months of age were treated intravenously with copper sulphate on 9th, 13th and 16th October, 1958. On each occasion the dose rate was 250 ml of 0·125 per cent. $CuSO_4 \cdot 5H_2O$ in physiological saline. On October 30, liver samples were taken for copper analysis and the animals were placed in the paddock to be mown subsequently for hay. Liver biopsies were taken at 3-weekly intervals up to January 28, 1959. The pasture was mown for hay on February 3. Intravenous copper therapy was repeated to ensure adequate liver copper reserves at the commencement of the stall-feeding trial.

For the stall-feeding portion of this experiment, the nine steers were distributed by stratified randomization on a body-weight basis to three groups each of three animals. The groups were allotted at random to the treatments as follows:—

Group I: Basal ration of 85 per cent. oaten chaff, 9 per cent. lucerne chaff, 6 per cent. linseed meal, plus sufficient copper sulphate to raise the copper content of the ration to that of the paspalum hay.

Group II: As for Group I, plus sufficient sodium sulphate to raise the inorganic sulphate level to that of the paspalum hay.

Group III: Paspalum hay.

All animals were fed the same amount of dry matter, which was slightly below the intake of the poorest eater.

Body-weight was recorded initially and at the conclusion of the 12 weeks of stall feeding.

Liver samples were taken initially and thereafter at 4-weekly intervals for the 12 weeks of stall feeding.

(b) Results

The average daily intake of copper, molybdenum and inorganic sulphate and the concentrations of these constituents in the diet of the experimental cattle are shown in Table 10. The copper level in paspalum hay, 6·5 p.p.m. Cu on dry-matter, was slightly greater than the mean value for the freshly cut paspalum fed in Experiment 3. The inorganic sulphate concentration of 0·35 per cent. was similar to that of the freshly cut paspalum used in the previous experiment. The molybdenum content of 0·1 p.p.m. Mo was even lower than that of the basal ration for Groups I and II.

Table 10

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF CATTLE IN EXPERIMENT 4

Group	Tag	Concentration in Diet			Mean Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	a	6·5	0·2	0·02	23·6	0·8	0·79
	b				23·6	0·8	0·79
	c				23·6	0·8	0·79
II	d	6·5	0·2	0·35	23·4	0·8	13·6
	e				23·6	0·8	13·8
	f				23·6	0·8	13·8
III	g	6·5	0·1	0·35	23·6	0·4	13·8
	h				23·6	0·4	13·8
	i				23·6	0·4	13·8

Changes in liver copper concentration in the nine experimental steers grazing the pasture from which the hay was subsequently made are shown in Table 11. All animals showed a marked and consistent fall in liver copper reserves on this pasture for the 12 weeks immediately prior to the time in which the paddock was mown for hay.

Table 11

COPPER IN LIVER OF CATTLE GRAZING THE PASTURE UP TO THE TIME OF MAKING HAY

Tag	Copper Concentration (p.p.m. Cu)					
	30.x.58	20.xi.58	11.xii.58	6.i.59	28.i.59	Change
a	233	156	122	86	51	−182
b	286	209	149	83	66	−220
c	207	150	119	60	29	−178
d	227	168	137	97	61	−166
e	212	134	97	44	29	−183
f	215	168	143	62	35	−180
g	238	179	152	89	51	−187
h	258	193	141	106	65	−193
i	192	136	89	50	28	−164
Mean	230	166	128	75	46	−184

Changes in body-weight and liver copper concentration of the cattle during the stall-feeding period are shown in Table 12. Cattle tended to make slight weight gains in all groups. Animals in Groups I and II showed slight reductions in liver copper levels during the 12 weeks on stall feeding, whereas greater falls were recorded in animals from Group III. There appears to be a factor in this paspalum hay which interferes with copper metabolism, and as the molybdenum content of this hay is even lower than in the basal ration this factor does not appear to be molybdenum in the presence of inorganic sulphate.

Table 12

BODY-WEIGHT AND COPPER CONCENTRATION IN LIVER OF STALL-FED CATTLE IN EXPERIMENT 4

Group	Tag	Body-weight				Copper Concentration (p.p.m. Cu)					
		Initial	After 12 Weeks	Change		Initial	After 4 Weeks	After 8 Weeks	After 12 Weeks	Change	
				Total	Group Mean					Total	Group Mean
I	a	560	584	+24		184	177	..	176	− 8	
	b	592	616	+24	+29	142	136	118	119	−23	−29
	c	622	658	+38		138	136	109	112	−26	
II	d	542	570	+28		224	236	204	220	− 4	
	e	624	646	+22	+20	154	132	117	125	−29	−11
	f	600	610	+10		244	247	..	243	− 1	
III	g	588	596	+ 8		143	104	94	86	−57	
	h	600	586	−14	+ 5	175	156	117	130	−45	−56
	i	636	658	+22		182	161	136	117	−65	

VII. DISCUSSION

The essential findings on sheep from Experiments 1 and 2 are:—

(1) On a basal diet of oaten chaff plus limited lucerne chaff sheep were able to at least maintain liver copper levels in spite of the low intake of copper.

(2) The addition of molybdenum to this diet of low inorganic sulphate content markedly increased blood molybdenum levels but did not interfere with the metabolism of copper by sheep.

(3) The addition of molybdenum to the diet in Experiment 2, which contained additional protein in the form of linseed meal, did interfere with the metabolism of copper by sheep.

(4) The addition of inorganic sulphate to the diet lowered blood molybdenum levels, allowed molybdenum to interfere with the metabolism of copper by sheep on the low protein diet of Experiment 1, and enhanced the molybdenum interference with copper metabolism in sheep on the higher protein diet of Experiment 2.

These findings are in agreement with those of Dick (1953, 1956).

In Experiment 2, sheep and cattle were fed similar rations. A comparison of the data shows that for the limited diets tested a similar copper/molybdenum/sulphate relationship is apparent in both species.

The rapid rate of decline in the liver copper status of cattle on pastures at Rocklea is shown in the data on grazing animals in Experiment 3. This rate of decline was greater in grazing cattle than in similar animals fed freshly cut pasture. Moir (1960) showed that grazing cattle on this property selected a diet containing 12 per cent. crude protein when the total available pasture contained 7·9 per cent. protein. The body-weight data in Experiment 3 lend further support to the selectivity of grazing cattle. Grazing cattle tended to gain weight while similar animals, stall-fed with freshly cut pasture, lost weight.

A number of reasons can be advanced to explain the difference in copper status between stall-fed and grazing cattle:—

(1) The animal gaining in body-weight could have a greater requirement for copper.

(2) The weight of liver, being proportional to body-weight, might be expected to be greater in the animal gaining weight than in the animal losing weight. If the same amount of copper was metabolized, a lower concentration of copper could occur in the liver of the heavier animal.

(3) The copper content could be lower in the selected diet.

(4) The copper could be less available in the selected diet.

The first reason seems unlikely to fully explain the difference. Cattle in Experiment 2 on a diet containing 4 p.p.m. Cu and 0·2 p.p.m. Mo gained more than 1 lb per head per day and remained in copper balance.

The second reason also appears inadequate. Body-weight gains in the grazing cattle in Experiment 3 were less than 4 per cent., whereas the decline in liver copper levels in these animals was more than 30 per cent.

Unpublished data on pastures on this property do not indicate a lower content of copper in the selected diet. There is little variation in copper content between pasture species in this sward, and new growth, which appears to be preferentially selected, tends to show a higher copper content. Further, the copper content of the total pasture available in Experiment 3 is 5·9 p.p.m. Cu; cattle in Experiment 2 tended to remain in copper balance on a compounded ration containing 4 p.p.m. Cu.

The fourth reason, availability of copper in selected pasture, must be regarded with favour until further evidence becomes available.

In Holland, van der Grift (personal communication) has found that hay made from pasture on which cattle are unable to maintain liver copper reserves usually provides sufficient available copper for animals to at least remain in copper balance. In Experiment 4 the hay-fed animals showed a decline in liver copper levels. However, the rate of decline was much less in the hay-fed group during the 12 weeks of stall feeding than in the same animals during the previous 12 weeks, when they were grazing the pasture from which the hay was made.

In the stall-feeding portion of Experiment 4 the decline in liver copper levels was greater in animals fed paspalum hay than in comparable animals fed a ration compounded from oaten chaff, lucerne chaff and linseed meal. The concentration and daily intake of copper and inorganic sulphate were the same but the molybdenum level in the hay was even lower (0·1 p.p.m. Mo) than in the compounded ration (0·2 p.p.m. Mo). Thus molybdenum in the presence of inorganic sulphate cannot be incriminated as the factor in the paspalum hay which interferes with the metabolism of copper by cattle.

From Experiment 3 it would appear that the addition of inorganic sulphate to the maintenance diet of oaten chaff, lucerne chaff and linseed meal caused a further decline in the liver copper levels of cattle although the molybdenum content of the diet was low (0·2 p.p.m. Mo). This did not occur in Experiment 4 when the same level of inorganic sulphate was added to a diet slightly better than maintenance containing different proportions of oaten chaff, lucerne chaff and linseed meal. No satisfactory explanation is apparent for this anomaly.

The findings from the experiments reported in this paper are in agreement with published data on the ability of molybdenum in the presence of inorganic sulphate to interfere with the metabolism of copper by sheep and cattle. The

results also support the conclusion that the factor or factors which interfere with the metabolism of copper by cattle grazing the predominantly paspalum pasture at the Animal Husbandry Research Farm at Rocklea is not molybdenum in the presence of inorganic sulphate.

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VIABLE HYPOTRICHOSIS IN A JERSEY CALF

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Various degrees of congenital hairlessness have been seen in cattle, and the literature on this subject has been reviewed by Hutt and Saunders (1953), who have proposed a classification of five categories. Semi-hairlessness in grade Hereford cattle in Queensland has been described by Young (1953). Our paper describes a case of congenital hairlessness in a Jersey calf which we believe to belong to the class "viable hypotrichosis" of Hutt and Saunders.

DESCRIPTION

The calf (Figure 1) was reported to one of the authors by Mr. J. H. Smith, Departmental Dairy Officer at Cooroy. It was a heifer aged four weeks by a grade Jersey bull out of a grade Jersey cow. As far as could be determined the sire and dam were unrelated.

Fig. 1.—Calf at 4 weeks of age.

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Hairlessness.—The calf was completely hairless except for the following areas:—

(1) *Ears.*—Hairs were present on the internal surfaces on the anterior border of the pinnae and on the proximal attachments of the posterior border of the pinnae of both ears.

(2) *Eyes.*—A few reddish eyelashes and a few eyebrow hairs were seen.

(3) *Muzzle.*—Adjacent to the upper and lower lips the usual vibrissae were present but only occasional coat hairs were visible.

(4) *Tail.*—The distal half of the tail was covered by greyish hair forming a tail switch.

(5) *Body.*—Hair density on the ventral aspect of the thorax and abdomen approached normal. The latter was moderately well covered as far laterally as the cutaneous muscle of the flanks. The hair on the thorax was fawn and extended over the lateral aspect of the ventral surface but not over the anterior part of the brisket. There were scattered hairs about the labia of the vulva.

(6) *Limbs.*—Scattered hairs were present around the pasterns, coronets and hocks of all four legs. The hooves were normal and fawn coloured.

Pigmentation.—The skin showed black pigmentation around the muzzle and lips but a light brown pigmentation was present in the skin over the head, neck and lateral aspects of the thorax and abdomen.

Teeth.—There was a normal complement of teeth, the calf having four incisors and four molars in each mandible and three molars in each maxilla.

Necropsy Findings.—The calf died during the night after transportation to the laboratory and a necropsy was conducted the next morning. The calf was in poor condition (weight 41 lb at 4 weeks). Apart from the hairless areas described above, no macroscopic changes were seen. The viscera were normal.

Histological Findings.—Histological examination of the liver, lung and thyroid did not show any abnormality. Apart from the skin section from the upper lip, which showed a few scattered normal follicles as well as the defective ones, 24 skin samples selected from various parts of the body and limbs showed the same picture of uniformly hairless follicles.

The most striking feature was the shallowness of the follicles, which penetrated only to the level of the sebaceous glands in the papillary layer of the dermis instead of extending into the reticular layer as is normal (Figure 2). Most of the follicles (Figures 3-4) were U-shaped and shallow, and all lacked the dermal papillae usually seen projecting into follicles. Their walls showed little differentiation but contained concentric layers of keratin derived from cornification

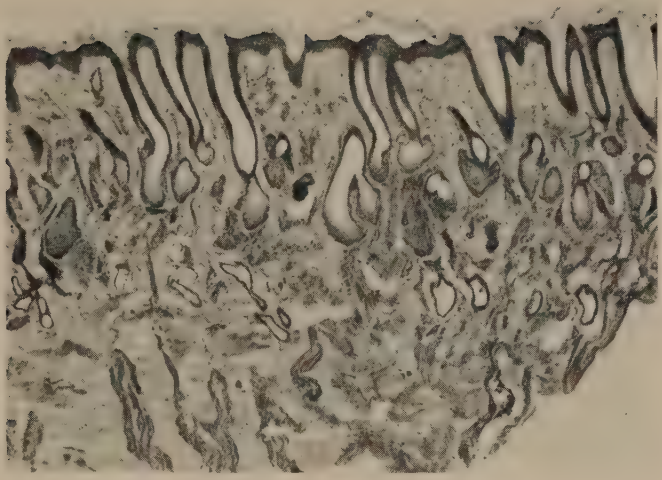
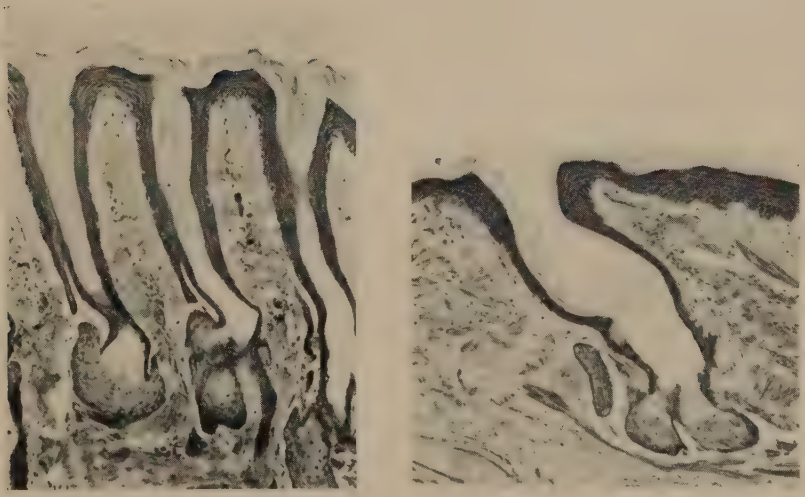


Fig. 2.—Skin from upper lip near muco-cutaneous junction. Note the shallow follicles. Haematoxylin and eosin. X50 (X16⅔, enlarged 3 times. Wratten filters Nos. 11 and 15).



Figs. 3 (left) and 4 (right).—Skin from middle of lateral surface of metacarpus. The follicles extend only to the sebaceous glands. X150 (X75, enlarged twice. Wratten filters Nos. 11 and 15).

of the walls. This keratin was continuous with the thin layer which covered the skin and appeared macroscopically as a thin scurf. Comparison with skin sections from apparently normal animals of approximately the same age indicated that there was no significant reduction in follicle density.

Sweat glands and sebaceous glands were plentiful and the former were quite prominent (Figure 2), although unlike the case reported by Hutt and Saunders (1953), they were not over-distended or cystic.

DISCUSSION

Hutt and Saunders (1953) attempted to systematize the different types of congenital hypotrichosis, and the following is a brief resumé of their classification:—

(1) *Lethal Hairlessness.*—The calves are born alive at full term but die within a few minutes of birth. Hair is confined to clearly demarcated areas, viz. muzzle, eyelids, ears, tail and pastern, but in the hairless areas the development of the hair follicles is arrested at an early stage. Teeth and hooves are normal. The condition, which has been seen in Holstein-Friesians in Sweden and Germany (Eisele 1936) and in Japanese cattle (Shibata and Ishihara 1949), is considered to be due to a simple autosomal recessive gene in the homozygous state.

(2) *Semi-hairlessness.*—At birth calves have a thin coat of short, fine, curly hair but later develop coarse, wiry hair which is not present in normal amounts. Craft and Blizzard (1934) and Kidwell and Guilbert (1950) showed that a simple recessive autosomal mutation caused this defect in Poll Herefords. No histological examination appears to have been carried out.

(3) *Hypotrichosis with Anodontia.*—At birth these calves lack hair (including eyelashes) and teeth but later develop some hair. Drieux *et al.* (1950) considered this defect was due to a sex-linked recessive gene. Histologically, there is malformation of the dermal papillae and cystic degeneration of the sweat glands, which lack excretory tubules.

(4) *Viable Hypotrichosis.*—Where the hairlessness is intermediate between Class 1 and Class 3 and hair is confined to inner surface of ears, eyelids and extremities of tail and legs. In Hutt and Saunders' (1953) case, microscopic examination of the skin showed hair follicles which lacked dermal papillae and which showed poor differentiation of the cells of the follicle wall. Sweat glands were cystic. This defect is transmitted as a simple autosomal recessive character.

(5) *Miscellaneous.*—Hutt and Saunders (1953) placed the defect of the hair and incisor teeth in Holstein-Friesians noted by Cole (1919) in this class.

Our case fits well into the class "viable hypotrichosis" as defined by Hutt and Saunders' (1953).

Hair was confined to the areas they describe and the histological picture (except for the absence of cystic sweat glands) was the same as described in their case. The absence of hair growth is due to the malformed hair follicles, which lack dermal papillae and the germinal area which secrets hair. As Hutt and Saunders' case was 10 weeks of age and ours was only 4 weeks it is possible that the difference in the sweat glands was due to age. It is possible that keratin plugs may have blocked the orifices in the older animal.

Many authors have indicated that these types of developmental anomalies are of genetic origin. No precise information could be gleaned about the hereditary background of our case so that evidence of genetic origin could not be given. The majority of cases of hypotrichosis appear to be produced by simple, recessive, autosomal genes and it is probable that the present case was produced in a similar fashion. If the cases are indeed caused by the same gene, then it appears to be widespread, having been reported previously in Jersey, Guernsey, Holstein-Friesian, Poll Hereford, Hereford and Ayrshire cattle.

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PROTEOLYTIC ORGANISMS IN BUTTER

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SUMMARY

Colonies producing zones on milk agar plates of routine butter samples were examined and it was found that in 55 per cent. of the zones, casein was not precipitated by tannic acid.

Organisms showing zone formation were isolated and examined for proteolytic properties. Approximately 20 per cent. of cultures showing proteolysis on milk agar were not caseolytic on casein agar, in casein broth or in milk.

Many organisms which were proteolytic were found to be heat-resistant.

In this series of cultures there was no relationship between proteolysis and lipolysis.

Only three cultures of Gram-negative rods were isolated among the proteolytic types. The remaining 47 cultures studied in detail were Gram-positive cocci and Gram-positive rods.

Statistical analysis of results for a large number of routine butter samples showed a relatively high correlation between total bacterial count and casein digester count and also between casein digester count and the presumptive coliform test.

It was concluded that there was no advantage in including a test for proteolysis on milk agar in the routine bacteriological tests for butter.

I. INTRODUCTION

Many workers (e.g. Hood and White 1928; Claydon and Hammer 1939; Pont 1941) have shown that certain flavour defects in butter, particularly those described as rabbito, putrefactive or surface taint, are caused by proteolytic organisms. Bacteria belonging to the genus *Pseudomonas* were found to cause these taints and the importance of water supplies as the source of these types has been emphasized by several investigators (Sorensen 1940; Turgasen 1940; Itzerott 1941). In addition to the above particular butter defects, some workers found that proteolysis by certain bacteria was responsible for off-flavours and lowered flavour scores (Spitzer, Parfitt, Manhart, and Epple 1927; Spitzer and Parfitt 1929; Elliker 1945). The presence of large numbers of lipolytic bacteria is also undesirable and can lead to the development of rancidity. When the effects of certain factors on the keeping quality of butter were studied by Guthrie, Schieb, and Stark (1936), they found that, in the absence of other factors, a direct correlation seemed to exist between the numbers of fat-splitting and casein-digesting bacteria present and spoilage of butter held at temperatures which permit bacterial growth.

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Stark and Schieb (1936) made an extensive investigation of fat-splitting and casein-digesting bacteria isolated from butter. These workers found that 96 per cent. of their cultures split tributyrin, 77 per cent. split fat and 63 per cent. were proteolytic on milk. These results suggest that determination of the numbers of lipolytic bacteria would give the best indication of the presence of undesirable lipolytic and proteolytic types. However, because of the toxicity of tributyrin and Nile blue sulphate, Stark and Schieb suggested that the use of milk agar to determine casein-digesters was important as an indication of keeping quality of butter.

A count of casein-digesting bacteria, intended to give an indication of the presence of potential fault-producing types, has been made regularly in the bacteriological analysis of samples for the Queensland Butter Improvement Service (Muller and Nichols 1950). The present investigation was undertaken to determine the types of bacteria which are found to produce zone formation on milk agar plates of butter samples, and to obtain information about their proteolytic and lipolytic properties.

II. DERIVATION OF ORGANISMS

Determination of the type of zone formation on milk agar was made initially on plates of samples of commercial salted butter which had been examined bacteriologically under the Queensland Butter Improvement Service. The zones produced were of two types: those which did not become cloudy when the agar was acidified by flooding the surface with 1 per cent. tannic acid, and those in which the casein was precipitated by the tannic acid. Colonies showing a zone not precipitated by a protein precipitant are normally considered to be proteolytic types.

In a series of 256 samples from 35 factories, 1,112 colonies producing zones were counted and the type of zone determined. In order to study in detail the properties of cultures producing zones, 74 such cultures were isolated from samples of butter from 22 factories. Fifty of these cultures produced zones not affected by tannic acid, and 24 produced zones precipitated by the acid.

In addition to the above types, 23 colonies which did not show zones on milk agar were isolated and purified. These cultures were obtained to compare the physiological properties of bacteria present in butter which did not affect the casein on milk agar plates.

III. METHODS

(a) Reaction on Milk Agar

In the routine bacteriological analysis of butter under the Queensland Butter Improvement Service, the total bacterial count has been made on milk agar, i.e. nutrient agar to which 5 per cent. sterile skim-milk has been added. A count

of casein-digesting organisms has been obtained by counting those colonies showing zone formation on the milk agar. Yeast and mould counts have been made on a synthetic yeast and mould medium, and a presumptive coliform test performed on 1/10 g butter in McConkey broth.

Purified cultures were stored on nutrient agar slopes, and nutrient broth was used for carrying cultures being tested.

Milk agar plates were poured, as in the routine testing, by adding 0·5 ml sterile skim-milk and 10 ml nutrient agar to the plate. Plates were incubated at 32°C and incubation for all tests with the exception of gelatin liquefaction was at this temperature.

Gram stains were made on 18 hr broth cultures of the organisms, using the Hucker modification of the Gram staining technique. Motility was determined from hanging-drop preparations of young cultures and the morphology from smears of skim-milk cultures, stained by Newman's stain.

Cultures were tested for heat resistance by holding at 65°C for 30 min, 1 ml of a young broth culture being sealed in a glass ampoule and completely immersed in water in a thermostatically controlled water-bath. The contents of the tube were plated on nutrient agar to determine survival after the heat treatment.

(b) Tests for Proteolysis

In addition to their reaction on milk agar, cultures were examined for hydrolysis of gelatin, proteolysis on casein agar, putrefaction of litmus milk, and casein breakdown in skim-milk, milk broth and liquid casein medium.

Gelatin liquefaction was determined by incubation of stab cultures at 22°C for 21 days. Gelatin medium was originally made up with and without peptone. However, the presence or absence of peptone made no difference to the reactions of the 60 cultures tested on both media, and subsequently nutrient gelatin including peptone was used.

Casein agar was prepared in a manner similar to that described by Frazier and Rupp (1928*a*), using nutrient broth instead of beef infusion. The casein solution and 3 per cent. agar were tubed in 5 ml amounts and mixed immediately before pouring the plates.

Putrefaction of litmus milk was determined by smell after 21 days at 32°C.

The determination of proteolysis in liquid media was done by the method described by Hull (1947) to estimate tyrosine and tryptophane, except that 2 ml of culture and 3 ml of distilled water were used with the original concentration of trichloroacetic acid. It has been shown that small amounts of protein hydrolysis, not detected by conventional methods, can be detected in this way.

The blue colour developed was measured in a direct reading photoelectric colorimeter using Ilford No. 607 orange filter. Control tests on uninoculated media were made in all series of tests. A standard tyrosine curve was prepared, using solutions of various tyrosine concentrations so that the colorimeter readings could be converted to tyrosine equivalent.

Casein medium was prepared containing 2 per cent. casein, which was dissolved in alkaline solution. The pH was adjusted to 7, phosphate buffer added in 0·02 M concentration, then 0·3 per cent. yeast extract and 0·5 per cent. peptone added. Initially tests were also made on media without peptone, but as in the case of gelatin liquefaction, the presence or absence of peptone did not affect the proteolysis, and subsequently all tests were made on media containing peptone.

Tests were also made for casein breakdown in milk broth. To 5 ml nutrient broth, 0·25 ml skim-milk was added, thus giving the same concentration of milk as was used in milk agar.

Determination of tyrosine production in milk, casein medium, and milk broth was made after growth of the cultures in these media for 7 days at 32°C.

(c) Tests for Lipolysis

Hydrolysis of fat was at first determined by using nutrient agar containing butterfat stained with Nile blue sulphate (Knaysi 1941). However, some cultures, especially the Gram-positive cocci, showed little or no growth on this medium. This is in agreement with the results reported by Jones and Richards (1952) concerning the inhibitory effects of Nile blue agar. Cultures were subsequently tested for lipolysis by streaking on nutrient agar containing 0.5 ml of a 3 per cent. fat emulsion in 10 ml agar. The incubated plates were flooded with a 1 in 1500 aqueous Nile blue sulphate solution to detect hydrolysed fat.

Hydrolysis of tributyrin was determined on nutrient agar containing 0·02 per cent. tributyrin. The tributyrin was toxic to many of the cultures and even at the above concentration growth of some was very poor.

IV. RESULTS

Details of the results of all tests on the 97 cultures isolated are shown in Tables 1, 2 and 3, in which the cultures are grouped according to their reaction on milk agar.

(a) Zone Formation on Milk Agar

The type of zone formation was examined on routine plates of butter tested in the Butter Improvement Service. In this way 1,112 colonies showing zones on milk agar were taken. Of these, 610, or 55 per cent., had zones which were not precipitated when the plates were flooded with tannic acid.

Table 1

RESULTS OF TESTS ON CULTURES SHOWING ZONES ON MILK AGAR NOT PRECIPITATED BY TANNIC ACID

No. of Cultures	Gram Stain	Morphology	C.D. on Milk Agar	C.D. on Casein Agar	Lipolysis		Spore Production	Heat Resistance	Motility	Hydrolysis of Gelatin	Proteolysis of—		
					Fat	Tributyrin					Milk Broth	Milk	Casein
2	+	cocci	+	+	–	–	–	+	–	+	+	+	sl
4	+	cocci	+	–	–	–	–	+	–	+	+	–	–
1	+	cocci	+	+	–	–	–	–	–	+	+	–	–
1	+	cocci	+	–	–	–	–	–	–	+	+	–	–
2	+	cocci	+	+	–	+	–	+	–	+	+	–	–
3	+	cocci	+	+	–	+	–	–	–	+	+	+	+
9	+	cocci	+	+	+	+	–	–	–	+	+	+	+
2	+	cocci	+	–	+	+	–	+	–	+	+	–	–
1	+	cocci	+	–	+	+	–	+	–	–	+	–	–
5	+	rods	+	+	–	–	–	–	–	+	+	+	+
4	+	rods	+	+	–	–	–	–	–	+	+	sl	+
2	+	rods	+	+	–	–	–	–	+	+	+	–	–
1	+	rods	+	–	–	–	–	–	+	+	+	+	–
2	+	rods	+	+	–	–	–	+	+	+	+	–	+
5	+	rods	+	+	–	+	+	+	+	+	+	+	+
2	+	rods	+	+	–	+	–	–	+	+	+	+	+
1	+	rods	+	–	–	+	–	+	+	+	+	sl	–
3	–	rods	+	+	–	–	–	–	–	+	+	+	+
50			50	40	12	25		19		49	50	35	35

Table 2

RESULTS OF TESTS ON CULTURES SHOWING ZONES ON MILK AGAR PRECIPITATED BY TANNIC ACID

No. of Cultures	Gram Stain	Morphology	C.D. on Milk Agar	C.D. on Casein Agar	Lipolysis		Spore Production	Heat Resistance	Motility	Hydrolysis of Gelatin	Proteolysis of—		
					Fat	Tributyrin					Milk Broth	Milk	Casein
1	+	cocci	p	—	—	—	—	+	—	+	—		—
2	+	cocci	p	—	—	—	—	+	—	—	—	—	—
5	+	cocci	p	—	+	+	—	—	—	+	—	—	—
1	+	cocci	p	—	+	+	—	—	—	+	—	sl	sl
2	+	cocci	p	+	+	+	—	—	—	+	—	—	+
1	+	rods	p	—	—	—	—	—	+	+	—	—	—
3	+	rods	p	+	—	—	—	+	+	+	—	—	sl
3	+	rods	p	—	—	+	+	+	—	—	—	—	—
3	+	rods	p	—	—	+	+	+	+	+	—	—	—
2	+	rods	p	—	—	+	+	+	+	+	sl	—	—
24				6	8	16		14		19	6	1	7

Table 3

RESULTS OF TESTS ON CULTURES WHICH DID NOT SHOW ZONE FORMATION ON MILK AGAR

No. of Cultures	Gram Stain	Morphology	C.D. on Milk Agar	C.D. on Casein Agar	Lipolysis		Spore Production	Heat Resistance	Motility	Hydrolysis of Gelatin	Proteolysis of—		
					Fat	Tributyrin					Milk Broth	Milk	Casein
3	+	cocci	—	—	—	—	—	—	—	—	—	—	—
4	+	cocci	—	—	—	—	—	+	—	—	—	—	—
3	+	cocci	—	—	—	+	—	—	—	—	—	—	—
4	+	cocci	—	—	—	+	—	+	—	—	—	—	—
1	+	cocci	—	—	+	+	—	—	—	—	—	—	—
3	+	rods	—	—	—	—	—	+	—	—	—	—	—
1	+	rods	—	—	—	—	+	+	—	—	—	—	—
1	+	rods	—	—	—	+	—	—	—	—	—	—	—
3	—	rods	—	—	—	+	—	—	—	—	—	—	—
23			—	—	1	12		12		—	—	—	—

Seventy-four cultures showing zones on milk agar were isolated for further study. It was found that 50, or 68 per cent., of these gave zones not precipitated by tannic acid.

The types of cultures showing proteolysis can be seen from Table 1. It is to be noted that only 3 of 50 isolates were Gram-negative rods, and that 25 were Gram-positive cocci.

(b) Digestion on Casein Agar

The reactions of all cultures on the casein agar of Frazier and Rupp (1928*a*) are included in Tables 1, 2 and 3.

Only 40 of the 50 cultures which gave zones on milk agar not precipitated by tannic acid showed proteolysis on casein agar. The other 10 cultures were not caseolytic on the casein agar. On the other hand, 6 of the 24 cultures giving precipitated zones on milk agar attacked the casein agar. The cultures which did not affect milk agar also showed no caseolysis on casein agar.

(c) Liquefaction of Gelatin

Forty-nine of the 50 cultures which produced zones on milk agar not precipitated by tannic acid liquefied gelatin. In addition, 18 cultures which showed zones which were precipitated also liquefied gelatin. All cultures which had no effect on milk agar did not attack gelatin. It would therefore appear that cultures showing other proteolytic powers were able to liquefy gelatin, but that many cultures able to attack gelatin did not hydrolyse other protein substrates.

(d) Proteolysis of Milk, Milk Broth and Casein Medium

Cultures which were actively proteolytic produced large amounts of tyrosine from milk and casein medium. Amounts of over 200 μg per ml in the cultures were considered to be positive, and the levels were generally much higher than this. Values between 80 and 200 μg per ml were recorded as slight and less than this amount as negative. In testing for proteolysis by this method in milk broth, the amounts of tyrosine produced were always of a much lower order. The most actively proteolytic cultures gave values in the range of 100 to 150 μg per ml. The reason for this difference in levels was not apparent. In this series of tests, values of over 90 μg per ml were considered to be positive, between 60 and 90 μg slight, and less than 60 μg negative.

A summary of the number of cultures attacking the above substrates is shown in Tables 1, 2 and 3. From Table 1 it can be seen that all cultures which gave zones not precipitated by tannic acid showed proteolysis in milk broth, but only 35 of the 50 attacked milk and 35 attacked liquid casein medium. In the second group of 24 cultures which showed precipitated zones on milk agar, 7 attacked milk broth, 1 attacked milk and 7 attacked casein. The 23 cultures which did not show zone formation on milk agar did not attack the liquid substrates.

(e) Peptonization of Litmus Milk

Reactions of the cultures in litmus milk at 32°C were noted after 7 days, and the cultures were checked again for off-flavours after 21 days. Twelve of the 25 cultures of casein-digesting rods, and two of the 25 cultures of casein-digesting cocci, peptonized milk. These cultures were strongly proteolytic in all other tests. The other strongly proteolytic cultures rapidly reduced and clotted the litmus milk and no peptonization could be detected.

(f) Relationship Between the Various Tests for Proteolysis

Gelatin appeared to be the substrate most easily attacked by organisms having any proteolytic powers. With one exception, cultures which did not attack gelatin did not attack any other substrate. There was generally good agreement between results in liquid and on solid media. Cultures which showed digestion on milk agar also showed proteolysis in the milk broth medium. However, 10 of these cultures were not truly caseolytic, as they did not attack casein agar or casein broth. There was also good agreement between the results of tests on casein agar and production of tyrosine and tryptophane from casein broth. Only three cultures giving a positive reaction on agar did not show caseolysis in broth medium. Five cultures which showed caseolysis in casein broth and on casein agar did not produce detectable amounts of tyrosine and tryptophane from milk and two cultures showed proteolysis in milk but not in the casein media.

From the results of tests on the 24 cultures which gave zones on milk agar precipitated by tannic acid, it appeared that 6 were caseolytic, and 3 others attacked milk or milk broth slightly. The remainder did not show any proteolytic powers on these substrates though some attacked gelatin. It would appear that the zones produced by these 15 cultures initially on milk agar were due to solution of the casein and not to digestion.

Cultures which did not produce zones on milk agar did not show any proteolytic properties.

(g) Lipolysis

Lipolytic properties were tested, using butterfat and tributyrin as substrates. Twenty-two cultures split fat and tributyrin and another 32 split tributyrin but not fat: 55 per cent. of the fat-splitting cultures and 48 per cent. of the cultures splitting tributyrin were also proteolytic. Therefore in this group of cultures there was no marked relationship between lipolytic and proteolytic properties.

(h) Heat-resistance

All cultures were tested for their ability to withstand heating to 65°C for 30 min. A total of 45 cultures was heat-resistant, these comprising 19 showing permanent zones on milk agar, 14 showing zones precipitated by tannic acid, and 12 which did not affect milk agar. The heat-resistant types included 22 cultures of cocci and 10 Gram-positive non-sporing rods in addition to the 13 cultures of Gram-positive sporing rods.

The percentage of heat-resistant types among the cultures studied here was much greater than that found by Stark and Schieb (1936). From 486 isolations, these workers obtained only 34 sporing rods and 7 Gram-negative non-sporing rods which were found to be heat-resistant. These organisms were obtained, however, from sweet cream butter, the cream apparently not being pasteurized. The cultures investigated here were all obtained from butters made from heat-treated cream. Therefore, it was to be expected that the total flora should contain a higher percentage of heat-resistant types. It was found that an appreciable number of the proteolytic and lipolytic bacteria were among the heat-resistant forms.

(i) Relationship between Casein Digester Count and other Tests on Routine Butter Samples

A summary of the distribution of total bacterial count and casein-digester count in 1971 samples examined under the Butter Improvement Service over a period of 12 months is shown in Table 4.

Table 4

Realtionship Between Total Bacterial Count and Casein Digester Count in Routine Analyses of Butter Samples

Casein Digesters per ml ('000)	No. of Samples with Total Bacterial Counts in Various Categories ('000 per ml)					Total
	< 20	> 20 and < 50	> 50 and < 100	> 100 and < 300	> 300	
< 1	244	75	45	25	4	393
1 to 3	325	143	56	28	9	561
4 to 5	85	72	24	13	2	196
> 5 and < 10	48	106	39	18	..	211
> 10 and < 20	10	48	41	22	5	126
> 20	..	42	97	218	127	484
Total ..	712	486	302	324	147	1971

These figures have been analysed statistically to determine the relationship between the two counts. Class intervals for bacterial counts were taken to be 20, 40, 80, 160, 450, or in terms of logarithms to the base 2 at relative values of 1, 2, 3, 4, 5·5. On this scale the variance within classes for number of casein digesters was reasonably constant, the average value being 0·996. The mean bacterial counts are summarized in Table 5. If the casein-digester count classes are taken to be centred at equally spaced log. values, 1, 2, 4, 8, 16, and 32, the correlation coefficient between the two counts is 0·6513.

Table 5

STATISTICAL ANALYSIS OF THE RELATIONSHIP BETWEEN TOTAL BACTERIAL COUNT AND NUMBER OF CASEIN DIGESTERS

	No. of Casein Digesters per ml ('000)	Bacterial Count		Percentage of Samples > 100,000 per ml
		log.	Equivalent Count per ml	
a	<1	1·66	31·5	7·4
b	1–3	1·68	32·0	6·6
c	4–5	1·86	36·2	7·7
d	>5–<10	2·13	43·7	8·5
e	>10–<20	2·73	66·5	21·4
f	>20	4·02	162·1	71·3

Significant differences f >> e >> d >> c > b, a

The relationship between casein-digester count and results of the presumptive coliform test has also been determined for the same 1971 samples. The results of tests are shown in Table 6. A X^2 test gave a value of 130·0 (5 D.F.), indicating that these percentages were not homogeneous. This reflects the obvious increase in positive tests with increasing casein-digester counts.

Table 6

RELATIONSHIP BETWEEN CASEIN DIGESTER COUNT AND PRESUMPTIVE COLIFORM TEST

Casein Digester Count	No. of Samples	Percentage of Positive Coliform Tests
<1	412	16·3
1–3	577	18·9
4–5	202	21·3
6–10	216	29·6
11–20	117	32·5
>20	491	48·5

V. DISCUSSION

In this investigation, the zone formation on milk agar plates by cultures isolated from butter has been compared with their ability to hydrolyse gelatin and break down casein in liquid media. It appeared that proteolysis on milk agar was not directly related to casein breakdown in milk, in casein broth or on casein agar. Hammer (1948, p. 101) similarly noted that some organisms which are actively proteolytic on milk agar have only limited action when inoculated into milk. He suggested that this may be due to the abundance of sugar in the milk which the organisms use in preference to casein. However, such organisms in this study were not proteolytic in casein media in the absence of sugar. A similar result was obtained by Frazier and Rupp (1928 *a*), who found that cultures

which were not caseolytic broke down lactalbumin in milk and failed to give a postive test for tryptophane. Frazier and Rupp (1928 *b*) also found that some cultures which were caseolytic were able to decompose lactalbumin as well as casein and these workers suggested that such cultures may attack lactalbumin preferentially in milk.

In the tests reported here a few cultures were caseolytic on the casein agar but not in the casein broth. In the latter case the casein was in the form of sodium caseinate, whereas in the agar medium it was present as calcium caseinate. Frazier and Rupp (1928 *b*) found that some cultures attacked the calcium salt more readily and this effect could have been operative here.

A few cultures which produced zones on milk agar precipitated by acid were found to be caseolytic in other tests. This serves to emphasize the difficulty of determining whether or not digestion has taken place on milk agar. On some occasions casein was reprecipitated when the plate was flooded with acid but the zone did not become as dense as the background, suggesting that some caseolysis had taken place, and care had to be taken in reading the result. The interpretation of casein-digestion on poured plates with a mixed flora is complicated because the size of the zone and the clarity depend on the depth of the agar, whether the colony is a surface or a sub-surface one, and the density of colonies on the plate.

The types of organisms isolated in the present study and found to be proteolytic differed markedly from the distribution found by Stark and Schieb (1936). In the present study only 3 of 50 isolates were Gram-negative rods and half the total were Gram-positive cocci. This difference can be attributed to the fact that Stark and Schieb made their isolations from sweet cream, unsalted butter, the cream apparently not being heat-treated, whereas butter examined here was made from neutralized, vacreated cream.

When all isolates were tested for lipolytic properties it was found that 22 per cent. hydrolysed fat and 55 per cent. hydrolysed tributyrin. However, there appeared to be no definite relationship between proteolysis and lipolysis. All cultures hydrolysing fat were Gram-positive cocci. These types were found by Jones and Richards (1952) to be the dominant lipolytic flora in butter and milk.

In Queensland a count of casein-digesting organisms has been utilized for some time in the Butter Improvement Service scheme, its purpose being to give some estimate of the presence of potentially fault-producing types. It was also considered that this count would give an indication of contamination after processing, as many workers have shown that types of organisms frequently occurring in contaminated water supplies are proteolytic. The results obtained here, however, show that a count of casein digesters includes types of organisms which are not caseolytic and do not cause proteolysis in milk. It therefore seems that many types of organisms would be included which would not cause lowering of quality because of proteolysis.

It has also been shown here that a substantial proportion of the proteolytic organisms is heat-resistant. Heat-resistance in these studies was examined by holding in broth at 65°C for 30 min. Treatment of cream for buttermaking is now at much higher temperatures than this. However, because of the protective action of fat in the cream during heating, the short time of exposure of the cream to high temperatures, and the fact that many of the heat-resistant types were spore formers, at least some of these heat-resistant forms could arise from the cream. Therefore the casein-digesting count cannot be expected to give reliable information of post-pasteurization contamination. In any case, the presumptive coliform test has been used successfully to indicate contamination after heat treatment and there is no need to attempt to use another test for this purpose.

It has also been stated (Stark and Schieb 1936) that there is a high correlation between proteolytic and lipolytic properties and that therefore the use of milk agar to determine casein-digesters was important as an indication of butter keeping quality. However, a marked relationship between proteolytic and lipolytic properties was not found to exist for organisms isolated in the present study, so that a count of casein digesters would have little relation to potential deterioration due to lipolytic organisms.

A statistical analysis of the results for total bacterial count and casein digester count in routine examinations of a large number of samples of butter showed that there was a relatively high correlation between these two values. This indicates that samples with high plate counts can be expected to exhibit also high casein digester counts. There was a similar finding with regard to the relationship between the presumptive coliform test and the casein digester count. Butters with positive results in the coliform test were found to have relatively high casein digester counts.

In view of these results and of the other tests reported in this paper it would seem that there is little ground for retaining a casein-digester count on milk agar in bacteriological tests on butter. It should be adequate to take the total bacterial count and the presumptive coliform test, in conjunction with tests for yeasts and moulds to indicate the bacteriological quality, without recourse to a casein-digester count which is of doubtful significance. There does not seem to be any need to perform tests, as a routine procedure, for specific groups of organisms such as lipolytic organisms, proteolytic organisms, casein-digesting Gram-negative rods, and psychrophilic organisms.

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ROLE OF THE FAT GLOBULES IN THE INHIBITION OF CERTAIN STRAINS OF *STREPTOCOCCUS CREMORIS* IN PASTEURIZED MILK

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SUMMARY

Removal of the fat from pasteurized wholemilk either by centrifugation or by gravity resulted in a skim-milk fraction in which susceptible strains produced greater acidity than in pasteurized wholemilk but to a lesser extent than in sterilized milk.

The cream fraction was found to be markedly inhibitory to all strains and it has been possible to transfer this inhibition to sterilized skim-milk by the addition of cream. The amount of cream added, however, greatly modified the inhibitory level. It appears likely that two separate factors were operating in the inhibition brought about by cream. The first factor, occurring with high dilutions of cream, was active only against the susceptible strains, while the other, active at low dilutions of cream, was inhibitory to both groups of organisms. This factor was probably due simply to a shortage of bacterial nutrients.

Resuspension of cream in skim-milk and water followed by subsequent inoculation with susceptible and resistant strains showed that in both cases the susceptible cells rose with the fat globules and remained in the cream layer. The resistant strain on the other hand was uniformly distributed between both layers.

Prevention of milk creaming by constant rotation of the tests resulted in complete loss of inhibition, so that both resistant and susceptible strains produced similar quantities of acid.

The inhibitory factor was washed out of the cream fraction by three washings with sterile water. The inhibitory factors in skim-milk could be removed by adsorption onto the washed cream.

I. INTRODUCTION

Recently attention has been focussed on the correlation between the creaming of milk and the inhibition of certain organisms, including starters, when this phenomenon takes place. This observation was first made by Wright and Tramer (1957). The following year Keogh (1958) proposed the hypothesis that the effect of creaming was a physical one, removing the agglutinated organisms from the bulk of the milk. In view of these results there seemed to be some need to investigate the actual role of the fat globules in the inhibition of starters affected by cream rising. A study of the resulting inhibition whether real or apparent (i.e. lack of nutrients) was a natural outcome of such an examination.

Some of the starters used were "susceptible" to an inhibitory factor in wholemilk, but which displayed good acidity production when creaming was prevented by the addition of rennet or agar, and some which were "resistant" to this factor.

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In every instance, acid production of cultures was measured by the activity test (Anderson and Meanwell 1942). In this test the test medium is inoculated at the 1 per cent. level with starter and incubated for 6 hr at 30°C. Acidity was determined by titrating 9 ml with ·1N NaOH, using phenolphthalein as indicator.

II. FAT CONTENT AND THE INHIBITORY PROPERTY

The correlation between fat content and the inhibitory property has been studied from three angles:—(1) An examination of the acidity production by these starters in skim-milk; (2) an examination of these organisms when grown in milk to which various quantities of cream has been added; and (3) prevention of formation of a cream layer.

(a) Methods

Separation was accomplished in several ways.

For separation by centrifugation, a portion of milk which had previously been found to be inhibitory was centrifuged at 3,000 r.p.m. on a Christ angle-head Junior 0 centrifuge for half an hour. The cream layer obtained was carefully removed and the skim-milk tested for inhibitory properties. Separation was also effected using a small dairy separator. Inhibitory milk was heated to 37°C and then separated. The milk portion was retained.

In separation by gravity creaming, pasteurized milk was dispensed in separating funnels in 160 ml quantities and refrigerated for various lengths of time to allow the cream to separate. At the end of each time interval the volume of the cream fraction was measured and the fat content of the skim-milk fraction determined. The skim-milk was retained for testing.

In each of the above cases, activity tests were set up, using both susceptible and resistant strains, and acidity determinations recorded after incubation for 6 hr at 30°C.

Tests were also set up in sterilized skim-milk containing various concentrations of cream. Activity tests were then carried out, using both susceptible and resistant strains.

(b) Results

It is apparent from the results in Table 1 that centrifugation and machine separation both gave a skim-milk which supported acidity production by the susceptible strains to a much greater extent than whole pasteurized milk. Both means of separation were equally effective in reducing the inhibitory potency. The resistant strains, however, produced approximately equal quantities of acid in either skim-mlik or wholemilk.

It appears also that allowing milk to separate by gravity resulted in a product which was somewhat less inhibitory than the original milk. There would seem to be a correlation between the degree of inhibition and the amount of fat present in the milk. The gravity-formed skim-milk obtained after 24 hours' storage contained the lowest concentration of fat and resulted in the highest degree of acidity formation by the susceptible strains.

Table 1

PERCENTAGE ACIDITY PRODUCED BY STRAINS OF STREPTOCOCCUS CREMORIS IN PASTEURIZED WHOLE-MILK AND THE SAME MILK PARTIALLY SKIMMED

(*a*) *Skim-milk obtained by centrifugation*

(1) By centrifuging in a Christ Laboratory centrifuge—3000 r.p.m.

Starter	Percentage Acidity in Wholemilk	Percentage Acidity in Skim-milk
HP (susceptible strain) ..	·30	·44
R6 „ „	·31	·47
C1 (resistant strain) ..	·56	·59
C13 „ „	·48	·47
Uninoculated control ..	·20	·19

(2) By passing through a small dairy separator

Starter	Percentage Acidity in Wholemilk	Percentage Acidity in Skim-milk
R6 (susceptible strain) ..	·28	·44
C13 (resistant strain) ..	·55	·56
Uninoculated control ..	·18	·18

(*b*) *Skim milk obtained by gravity separation of cream by storage at refrigeration temperatures for periods up to* 24 *hours*

Duration of Storage	Fat Content of Skim-milk Fraction	Volume of Cream Layer	Percentage Acidity in Skim-milk	
(hr)	(%)	(%)	Susceptible Strain R6	Resistant Strain C13
0	3·9	·6	·21	·44
2	3·9	5·0	·22	·44
3	3·4	5·0	·22	·44
4	3·2	5·0	·22	·44
5	3·1	5·0	·22	·44
6	3·1	5·75	·23	·44
24	2·4	6·25	·29	·44

This correlation between fat content and acidity production takes more point when it is considered that centrifugation resulted in milks containing approximately 0·4 per cent. fat.

An examination of Figure 1 shows a marked difference in the behaviour of resistant and susceptible strains in response to increasing cream concentration. When a small proportion of cream was added to sterilized skim-milk the susceptible strains were inhibited, but the resistant strains showed no effect. As the proportion of cream to skim-milk was increased, the susceptible strains were further inhibited, reaching a maximum degree of inhibition (minimum acidity production) at 10 per cent. The maximum was maintained until 25 per cent. concentration of cream had been attained. With further increases in the proportion of cream the degree of inhibition with the susceptible strain was reduced and was at its lowest at 75 per cent. cream. In the case of the resistant strain, increases in cream concentration produced first a slight stimulation, then inhibition, and finally marked inhibition at concentrations exceeding 75 per cent. When the concentration of cream reached 100 per cent., both susceptible and resistant strains were equally and strongly inhibited. These results therefore show that, under the conditions of the experiment, the degree of inhibition changed with the percentage of cream in the mixture.

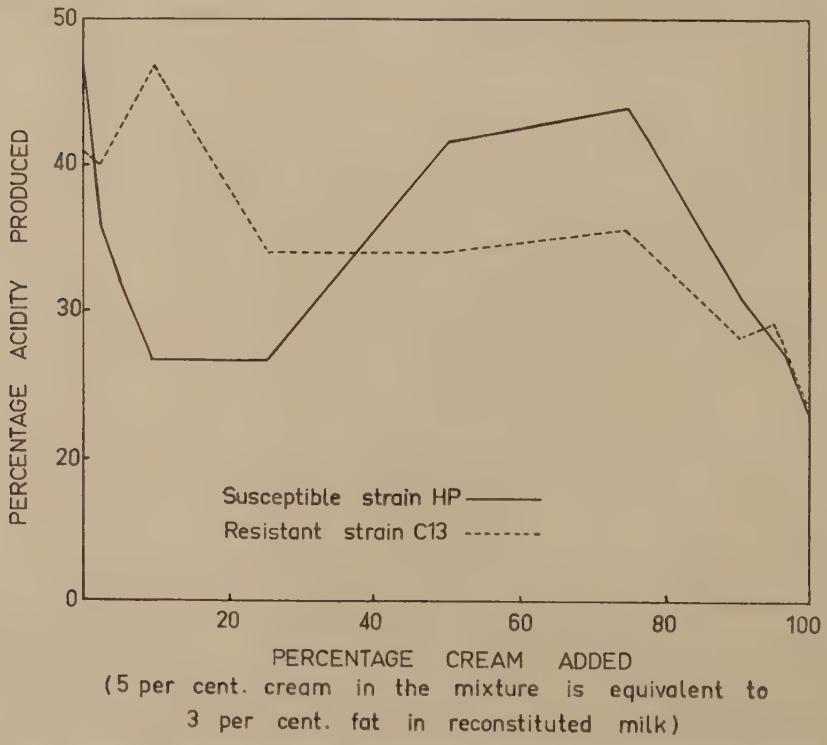

Fig. 1.—Effect of increasing cream concentration on resistant and susceptible strains.

It is pointed out that additions of cream at a level of 5–8 per cent. to sterilized skim-milk yields milk with a fat content approximating that of normal milks.

III. PREVENTION OF MILK CREAMING BY CONSTANT ROTATION OF TESTS

Wright and Tramer (1957) were able to show that homogenization and inversion of the tubes every 30 min resulted in increased acidity production. An experiment was therefore devised to completely prevent the formation of a cream layer.

(a) Methods

Two 100 ml portions of pasteurized milk were inoculated at the 1 per cent. level, one with a susceptible strain and the other with a resistant strain. After thorough mixing each milk was pipetted into a wide-necked 1 oz McCartney bottle so that each bottle was completely filled. In order to prevent inclusion of air at the top of each container, a rubber stopper fitted with a piece of glass tubing and stopcock was placed in the neck of each bottle. The excess milk then flowed into the glass tubing, carrying away any residual air. After all the tubing was filled with milk the stopcock was closed. The stoppers were then securely tied down to the bottles. Both bottles were clamped down to a revolving spindle travelling at 10 r.p.m. Because of the absence of an air bubble, no agitation of the milk occurred during inversion. Both samples were allowed to rotate for 6 hr at room temperature (26-28°C). Another two bottles were set up as controls without constant inversion. At the end of 6 hr, smears were made and the acidity percentage of each milk determined.

(b) Results

The results of two separate tests are set out in Table 2.

It is obvious from these results that inhibition of the susceptible strains occurred only when a cream layer was allowed to form on the top of the milk and not when the test bottles were constantly inverted to prevent creaming.

Table 2

ACIDITY PRODUCTION OF "SUSCEPTIBLE" AND "RESISTANT" STRAINS IN MILKS ALLOWED TO CONSTANTLY ROTATE DURING A 6-HOUR INCUBATION PERIOD AT ROOM TEMPERATURE (26–28°C)

	Susceptible Strains		Resistant Strains	
	HP	R6	C13	C3
Bottles constantly revolving to prevent formation of cream layer	·54	·40	·44	·45
Bottles allowed to stand to facilitate the formation of cream layer	·22	·22	·40	·47

Smears made from one set of tests (using HP and C3) which had been allowed to rotate revealed that the susceptible strain (HP) was agglutinated and that large numbers of the organisms were congregated around the fat globules. It was also noted that individual chains were situated around fat globules. However, the resistant strain (C3) showed no evidence of agglutination. These results show that agglutination in itself is without effect on the susceptible strain. Inhibition is manifest only with the formation of a cream layer when the cells are taken up with the rising fat globules.

It is to be noted, moreover, that in the bottles constantly inverted, agitation and aeration of the milk did not occur because all air had been removed. In these tests the pasteurized milk had been stored in a refrigerator for 48 and 72 hr respectively prior to testing, so that clumping of the fat could be expected to have taken place.

The results obtained here agree with those of Wright and Tramer (1957) but go further in that external influences such as aeration and agitation are completely eliminated.

IV. ASSOCIATION OF "INHIBITORY" FACTOR WITH THE CREAM LAYER

There are indications that the factor causing the concentration of the susceptible strain in the cream layer must in part, at least, be associated with the fat globule; this assumption is supported by the fact that additions of cream to sterilized milk reduced the acidity production of the susceptible strains. Tests were therefore set up to determine whether the susceptible organism rose with the cream when resuspended in water.

(a) Methods

Pasteurized milk was collected and stored in the refrigerator for 24 hr prior to use. The milk was skimmed in a dairy separator and the cream and skim portions retained. Four sterile separating funnels were filled with 140 ml of sterile tap water and four with 140 ml of skim-milk; 14 ml of cream were added to each of the separating funnels. All funnels were inoculated with starter at the rate of 1 per cent., the contents thoroughly mixed and the plate counts done on each one immediately. The funnels were incubated for 6 hr at 32°C and the plate counts and acidity titrations were then performed. Two funnels from each pair were used as controls.

(b) Results

The results are set out in Tables 3 and 4.

Table 3

DISTRIBUTION OF STARTER ORGANISMS FOUND IN GRAVITY CREAM AND SKIM-MILK WHEN AN INOCULATED RECOMBINED MILK IS STORED FOR 6 HOURS AT 30°C

Strain	Pre-Storage Initial Count (x 10^6)	After 6 Hours Storage at 30°C				Percentage Volume Cream
		Percentage Acidity		Final Count (x 10^6)		
		Skim	Cream	Skim	Cream	
Susceptible Strain—R6 ..	1·4	·25	·36	20	162	2·8
Susceptible Strain—R6 ..	1·5	Milk after remixing—·25		Milk after remixing—6		
Resistant Strain—C3 ..	9·0	·68	·41	640	292	2·8
Resistant Strain—C3 ..	6·0	Milk after remixing—·55		Milk after remixing—492		

Table 4

DISTRIBUTION OF STARTER ORGANISMS FOUND IN GRAVITY CREAM AND THE AQUEOUS PHASE WHEN AN INOCULATED SUSPENSION OF CREAM AND WATER IS STORED FOR 6 HOURS AT 30°C

Strain	Pre-Storage Initial Count (x 10^6)	After 6 Hours Storage at 30°C				Percentage Volume Cream
		Percentage Acidity		Final Count (x 10^6)		
		Aqueous Phase	Cream	Aqueous Phase	Cream	
Susceptible Strain—R6 ..	·7	·04	·11	1·2	18	15
Susceptible Strain—R6 ..	1·1			After remixing 7		
Resistant Strain—C3 ..	7	·04	·11	18	21	15
Resistant Strain—C3 ..	8			After remixing 22		

It is evident that the susceptible strain was carried up with the rising fat whether it was suspended in skim-milk or in tap water. The resistant strain was nearly equally divided between the aqueous phase and the cream. However, the cream volume in water was approximately five times as great as in skim-milk. Another interesting feature of the results is that the susceptible strain behaved in exactly the same manner in cream and skim-milk recombined as in wholemilk.

Table 4 shows that the development of acidity in the cream layer was very slow for both susceptible and resistant strains. This result confirms that already obtained when both strains were grown in cream, and it seems probable that the growth of organisms in a complete medium of cream involves a separate investigation.

The explanation of the cream rising in tests where it was recombined with water seems to be due to the fact that the milk prior to skimming had been standing for 24 hr, which was sufficiently long for the fat globules to have clumped and risen to the surface of the milk. Once this has happened, it is probable that

even after stirring and skimming at 37°C the fat globules were aggregated together and therefore rose to the surface of the liquid whether the cream had been resuspended in milk or water.

It was found that if the milk was heated to 55°C prior to separation, the cream did not rise to form a layer on the top of the aqueous phase. This observation, of course, further confirms the conclusions of Sharp and Krukovosky (1939).

V. ELIMINATION OF THE INHIBITORY SUBSTANCE FROM CREAM BY WASHING

As the inhibitory factor of milk is associated with the fat globule, it seemed possible that a substance causing inhibition of the susceptible strains might be adsorbed on the fat globule membrane. Isolation of the fat globule membrane involves its separation from the aqueous phase of the milk (plasma proteins, lactose and salts) and from the material contained inside the fat globules. The first aim is achieved by washing the fat globules with water and the second by extraction with fat solvents. Preliminary investigations showed that the inhibitory factor could be separated from the cream merely by washing with water. This section contains a description of this process and its implications.

Several workers (Rimpila and Palmer 1935; Jack and Dahle 1937; and Brunner, Duncan, and Trout 1953) have shown that most of the milk plasma protein is washed out of the cream after the first washing. After the third washing practically all the plasma protein has been diluted out of the cream and any further lowering of the protein: fat ratio is insignificant. Rimpila and Palmer (1935) and Heinemann (1939) found that the decrease in the phospholipid content after the first washing is not so pronounced, being about 80 per cent. of the original content. After the third washing, however, both protein and phospholipid contents remain almost constant.

Palmer (1944) pointed out that the agitation and dilution effect involved in the washing of cream probably washes out all but the most tenaciously adsorbed materials from the fat.

It is noteworthy, also, that the standard methods for the isolation of membrane material, as reported by King (1955), stipulate six washings of the cream prior to further treatment for the isolation of the membrane material.

(a) Methods

Pasteurized milk was collected and separated by passing it through a small dairy separator at 37°C. The cream fraction was retained. This was washed by two methods as follows:—

(1) A portion of cream was diluted with an equal quantity of sterile tap water and both fractions were mixed by gently rotating the flask, which was then

heated to 37°C. The contents of the flask were then separated and both cream and the washings retained. A sub-sample of approximately 20 ml was taken from the cream and the remainder was again washed twice more in the manner described above. Sub-samples of the cream were again withdrawn after each of these washings. Each wash water was retained.

(2) A portion of the cream was diluted with an equal quantity of sterile tap water and placed in a separating funnel in the refrigerator. After a few hours a sharply defined cream layer had formed and the aqueous phase was withdrawn and retained. A sub-sample of cream was taken (to do this the cream had to be warmed to 37°C). The cream was washed twice more and sub-samples of the cream after each washing retained. Each wash water was also kept. It had previously been found that additions of cream from 10 to 25 per cent. to sterilized milk resulted in a maximum inhibition of a susceptible culture. Therefore both 10 and 25 per cent. additions of each cream were made to sterilized milk. At the same time, tests were set up wherein similar amounts of wash water were added to the sterile milk. All tests were inoculated with a susceptible strain (R6) at the 1 per cent. level and the usual activity tests performed.

Microscopic examinations were also made of the skim and cream layers of the tests containing the creams after successive washings, using long-chain strain R6 and short-chain strain R1. Smears were also made of the tests containing the washings, which were stained and examined. These smears were simply stained with Newman's stain.

(b) Results

The results are set out in Table 5. In this table, there are indications that the inhibitory factor can be washed out of the cream. One washing was insufficient to remove any of the inhibitory properties. However, after a second washing the cream appeared to be less inhibitory. The third washing resulted in a cream which when added to skim-milk caused stimulation of the susceptible strain at concentrations of cream at the 10 and 25 per cent. levels, although stimulation was not quite as marked with the test containing 25 per cent. of cream as with that containing 10 per cent.

The table also shows that the washings may contain the inhibitory properties when added to sterile skim-milk. The first washing was actually stimulatory, whereas the second contained inhibitory factors to a marked extent. The third washing, on the other hand, had no effect on the acidity production by the susceptible strain. Washings obtained by both methods gave similar results.

Microscopic examinations of washed creams following resuspension with sterile skim-milk and inoculated with susceptible strains confirmed the above results. The cream layer which formed when the cream after one washing only

Table 5

PERCENTAGE ACIDITY FORMATION OF A "SUSCEPTIBLE" STRAIN IN STERILE MILK CONTAINING (*a*) CREAM BEFORE AND AFTER EACH OF THREE SUCCESSIVE WASHINGS WITH STERILE TAP WATER AND (*b*) EACH CREAM WASHING

—	Percentage Acidity Susceptible Strain R6	—	Percentage Acidity Susceptible Strain R6
Sterile skim containing 25% cream ..	·28	Sterile skim containing 10% cream	·28
Sterile skim containing 25% cream (after 1st washing)	·28	Sterile skim containing 10% cream (after 1st washing)	·28
Sterile skim containing 25% cream (after 2nd washing)	·35	Sterile skim containing 10% cream (after 2nd washing)	·44
Sterile skim containing 25% cream (after 3rd washing)	·45	Sterile skim containing 10% cream (after 3rd washing)	·50
Sterile skim—	·40		
Sterile skim containing 25% .. 1st wash-water	·42	Sterile skim containing 10% .. 1st wash-water	·52
Sterile skim containing 25% .. 2nd wash-water	·26	Sterile skim containing 10% .. 2nd wash-water	·37
Sterile skim containing 25% .. 3rd wash-water	·40	Sterile skim containing 10% .. 3rd wash-water	·41
Sterile skim containing 25% sterile tap water—control	·40	Sterile skim containing 10% sterile tap water—control	·40

Fat Content First wash-water ·9% ; second wash-water ·25% ; third wash-water ·25%.

was used contained most of the organisms, but the skim-milk contained relatively few. However, when creams were used following the second and third washings, only a few organisms were found in the cream layer and the majority remained in the skim-milk. There was no evidence of agglutination of the short-chain strain in any of the tests but interpretation of agglutination with the long-chain strain was obscure.

VI. ELIMINATION OF INHIBITORY FACTORS FROM SKIM-MILK BY ADSORPTION ONTO WASHED CREAM

As the inhibitory factor may be washed out of the cream, it seemed likely that addition of such washed cream to skim-milk would result in some of the inhibitory factors present in the skim-milk being adsorbed onto the washed cream. This possibility is explored in this section.

(a) Methods

Milk was skimmed and both skim and cream fractions were retained. The cream was washed three times and separated after each washing. This washed cream was then added to the skim-milk and left in the refrigerator overnight. The following morning the milk was warmed to 37°C and separated. Both fractions were retained.

Activity tests were set up, using two susceptible strains. Acidity developed by each was estimated in the original skim-milk and skim-milk obtained after recombining with washed cream. Reconstituted milk was formed from the different combinations of skim-milk and cream.

(b) Results

The results are set out in Table 6. They indicate that the inhibitory factor in milk is adsorbed onto the fat globules. Such adsorption is reflected in the increased acidity production by both strains in skim-milk obtained after such adsorption had been allowed to take place. It appears also that inhibitory properties in the skim-milk primarily determine the acidity production of the recombined milk. However, as is seen by the recombination with the adsorbed cream, both fractions exert a cumulative action.

Table 6

DISTRIBUTION OF INHIBITORY FACTORS IN SKIM AND CREAM FRACTIONS AFTER RECOMBINING SKIM AND WASHED CREAM

Milk	Percentage Acidity Produced in Susceptible Strains	
	HP	R6
Skim-milk	·35	·31
Skim-milk A	·50	·40
Skim-milk containing 10% washed cream	·30	·35
Skim-milk containing 10% cream A	·28	·30
Skim-milk A containing 10% washed cream ..	·38	·37
Skim-milk A containing 10% cream A	·35	·31

Skim-milk A—obtained after recombining skim-milk with washed cream.
Cream A—obtained after recombining skim-milk with washed cream.

VII. DISCUSSION

It is of interest that skim-milk obtained by gravity and centrifugal separation was less inhibitory than the original product. This indicates that the inhibition has been partially removed with the fat.

On the other hand, as demonstrated by Wright and Tramer (1957), when milk was recombined following heat treatment of either or both fractions, it was the skim-milk fraction which determined the final degree of inhibition of the susceptible strains.

Jago (1954) was the first to note that the inhibitory substance was closely associated with the fat globule. However, he added cream at the rate of 1–5 per cent. to sterilized milk. He therefore did not discover the presence, shown herein, of a stimulatory zone followed by a second inhibitory level when increasing concentrations of cream were added. Both resistant and susceptible strains are affected by the second inhibitory zone, which is probably the result of a nutritional deficiency in the cream.

The presence of stimulatory factors in milk has been noted on a number of occasions. Jago (1954) found that separator slime was stimulatory to "susceptible" starter strains studied by him. Czulak and Meanwell (1951) merely presented an hypothesis that presence or absence of stimulatory substances in the milk determined "winter slowness" of cheese starters. Although in this present work it has been possible to stimulate susceptible strains in milk by addition of separator slime, it seems unlikely that sufficient of these substances would still be in the cream to account for the stimulatory level.

It is clear from the results that any change in the potency of the inhibitory factor in the milk parallels, at least in part, changes in the fat content of the milk. However, it is likely that some other factor is involved also. This is shown by the fact that violent agitation of inhibitory milk resulted in complete destruction of the inhibitory factor only after maximum degree of churning had been reached (Gillies 1959).

There appears to be little doubt that the physical state of the milk plays an important role in determining the inhibitory nature of pasteurized milk. Although Wright and Tramer (1957) would appear to be correct in this regard, their theory regarding the cause of inhibition seems to have little justification.

It is also noteworthy that constant rotation of wholemilk resulted in the removal of the inhibitory properties even though there was considerable evidence of gross agglutination of the susceptible strains. Therefore, if agglutination is concerned with the inhibitory phenomenon, it must only be when a cream layer is allowed to form.

Because the susceptible strains are congregated in the cream layer of unagitated wholemilk even after one hour (Wright and Tramer 1957), it is likely that the organisms are attracted to the surface of the fat globules as they rise to form the cream layer. Further proof of such an attraction is available, as the susceptible strain rose with the cream when suspended in water.

As it has been found possible to wash an inhibitory substance from the cream after the second washing with tap water or saline, it is impossible to consider such a substance as an integral part of the fat globule membrane. In the classical methods of examining the fat globule membrane substances, as many as six preliminary washings have been employed. It is likely that there is a loose linkage between the inhibitory substance and some component or components of the fat globule membrane, which linkage is destroyed by washing with tap water or isotonic saline. It seems probable that a milk serum protein is involved as an essential component leading to inhibition, this component becoming enmeshed with the fat globules as they rise to form a cream layer. It is clear, however, that some bonding stronger in nature than a mere enmeshment is involved, because marked inhibition was found to occur when cream was added

to sterilized skim-milk. In addition, simple enmeshment suggests that this material would be at least partially removed from the fat globule membrane by the first washing, whereas in fact it was not removed until the second washing.

It has been possible to transfer the inhibitory property to sterile skim-milk by the addition of cream washings. From these results it is possible to propose that inhibition is due to the presence of at least one chemical substance and is not a result of overcrowding or lack of nutrients when a cream layer is formed. As the fat content of the washings was as low as 0·2 per cent., it is impossible to correlate inhibition with the presence of a cream layer when such washings are added to sterile skim-milk. It is also of interest that there was no evidence of agglutination when a short-chained susceptible strain was grown in such mixtures. The latter observation indicated that agglutination is quite independent of this type of inhibition.

The inhibitory property of milk for the susceptible strains appears to be a complex one and is most evident when the organisms are grown in unagitated wholemilk. There are numerous indications that the inhibitory properties of milk are associated with both cream and skim fractions.

Skim-milk itself is much less inhibitory than wholemilk. Such a modification can be thought to be correlated with the great reduction in fat content. Nevertheless, there are indications that the skim fraction contains a potential inhibitor which determines the final degree of inhibition in wholemilk. There is much evidence for such an hypothesis. For example, the degree of heat treatment of the skim-milk fraction, not that of the cream, determined the degree of inhibition of the susceptible strains in whole recombined milk. These results were paralleled when washed cream was added to skim-milk.

On the other hand, there is ample evidence available demonstrating the inhibitory properties of the cream fraction. This is shown most dramatically when cream is added to sterilized milk and also by the fact that the inhibitory property can be removed from cream by mere washing with water. Further indications that the bacterial cells are attracted to the fat globules are provided when cream is resuspended in water. Such a mixture gives results which show quite definitely that the susceptible strains are attracted to the fat globules and that they will rise with the cream in the absence of skim-milk.

Although the presence of a cream layer is necessary for inhibition of a susceptible strain, the mechanism of inhibition is not clear. The cells of the susceptible strain rose continually as the cream layer formed, but the final bacterial numbers in the cream were comparable with those of the resistant strain. It is probable that agglutination was responsible for such plate counts in the cream layer. Other possible causes of lowered bacterial numbers are that the numbers of the susceptible bacteria in the cream layer exceeded the M-concentration, and

that the inhibition was associated with the unavailability of nutrients. Nevertheless, it is difficult to reconcile these theories with the fact that an active inhibitory factor was washed out of the cream and that this when added to sterile skim-milk was found to cause inhibition of the susceptible strains. It is also noteworthy that there was no evidence of agglutination when a short-chained susceptible strain was grown in such mixtures. Therefore, it is concluded that agglutination is not necessarily an integral part of inhibition but rather tends to accompany it.

To sum up: skim-milk would appear to donate a factor which combined with another on the fat globule membrane can result in inhibition if creaming is permitted. Both are necessary for maximum inhibition. Creaming if permitted allows maximum interaction between the two. The addition of cream washings to sterile skim-milk merely permits the interaction without the necessity of creaming.

VIII. ACKNOWLEDGEMENTS

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SEROLOGICAL PROPERTIES OF MILK AND THEIR ROLE IN REGARD TO THE INHIBITION OF CERTAIN STRAINS OF *STREPTOCOCCUS CREMORIS*

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SUMMARY

Serological aspects involved in the inhibition of certain strains of *Streptococcus cremoris* have been examined. The study has included an investigation into the presence of antibodies occurring naturally in milk as well as an examination of the effects of adding homologous antisera to milk containing either susceptible or resistant strains.

Whey agglutination tests showed that there were no antibodies present for the resistant strains, but tests carried out on susceptible strains were unsatisfactory because of the instability of the antigen.

A modified ring test gave positive results for the susceptible strains but was negative for the resistant strains. As the susceptible strains were found to be rough variants and the resistant ones were smooth, it is considered that the positive tests were due to non-specific agglutination.

Microscopic examination revealed that all susceptible strains were agglutinated in both pasteurized wholemilk and skim-milk. Only the long-chain resistant strains, however, showed evidence of agglutination. Long-chain susceptible strains exhibited a tendency to agglutinate in sterilized milk, but short-chain susceptible strains did not. Such agglutination in itself had little effect on acidity production.

Addition of homologous antiserum to pasteurized milk inoculated with a resistant strain resulted in it behaving in all respects like a susceptible strain in pasteurized milk.

By adding homologous antibody to recombined milk (sterile skim-milk and heated cream) inoculated with a susceptible strain, it was shown that the addition of specific antibody caused the susceptible strain to react in the same way as in whole separated milk. However, in this case the resulting inhibition was not quite so marked.

Inoculation of susceptible strains into sterile skim-milk containing cream washings gave results which showed that the inhibitory property was transferable. It was also found that when a short-chain susceptible strain was used there was no evidence of any agglutination of the starter cells.

Conclusions have been drawn from the data obtained and an explanation for the inhibition of the susceptible strains has been advanced. It appears that both skim-milk and cream fractions donate factors which separately cause inhibition of acidity production by the susceptible strains but both these factors are required for maximum inhibition.

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I. INTRODUCTION

Over the past 60 years, numerous observations have been made regarding antibodies in milk and their possible link with the inhibitory properties of milk. Ehrlich (1892) discovered that antibodies of various types may be found in milk. Marrack (1947) reported that the young animal depends on the antibodies in colostrum or milk for its passive immunity. He quoted that a number of investigators, including Little and Orcutt (1922) and Mason, Dalling, and Gordon (1930), agree that colostrum is the main source of antibodies. As lactation advances, the antibody level appears to fall away. Campbell, Humphrey, and Work (1953) were able to demonstrate by using labelled methionine that antibodies are transferred directly from blood to milk.

Heinemann and Glenn (1908) and Rosenau and McCoy (1908) found that *Salmonella typhi* was agglutinated in milk, and they thought that agglutination was responsible for the apparent decrease in the number of bacteria in fresh milk. Heinemann and Glenn were also able to show that species from several genera were agglutinated; they therefore thought this agglutination was probably unspecific, a finding which is verified in the present work. Chambers (1920), however, was unable to find a correlation between agglutination and bacterial inhibition. In support of this later finding, Jones and Little (1927) by various means were able to rule out the action of agglutinin on a non-haemolytic mastitis Streptococcus. They found that all milks inhibited this Streptococcus. More recently, Hobbs (1939) presented evidence that there are two inhibitory factors in milk, the first responsible for the clumping of the growing organism and the second for the bacteriostatic effect which inhibits bacterial multiplication for a variable length of time. Wright and Tramer (1957) noted that cheese starters affected by cream rising in wholemilk were agglutinated. However, as they presumed this agglutination was also present in homogenized or separated milk which was no longer inhibitory, they concluded that agglutination in itself had no effect on acidity production. This finding was confirmed by Keogh (1958).

It is of interest also that Morris and Edwards (1949) found that cultures of *Streptococcus lactis* varied considerably in morphology when grown in raw and in heated milk. They considered this morphological variation to be the cause of the different colony counts obtained with the two types of milk. These workers showed that the clumps formed were due to the proliferation of the growing organism, and were not a result of agglutination. Wright and Tramer (1957) also noted a difference in chain length of the slow starters, the chains being longer in pasteurized milk than in milk heated to higher temperatures. However, the latter pair of workers did not give any information regarding nomenclature of the starters.

From the foregoing evidence it is clear that further examination of the serological role in the inhibitory phenomenon was necessary. Such an examination has been made in the present work. There were two main aspects of the investigation, viz:—

(1) The presence of antibodies in inhibitory milk.

(2) The effects produced on starter streptococci by the addition of homologous antisera.

Throughout the work the starters used were strains of *Streptococcus cremoris*. These were labelled (*a*) "susceptible" (e.g. HP and R1, which were inhibited in whole, raw or pasteurized milk allowed to stand but produced acidity normally in homogenized or sterile milk) and (*b*) "resistant" (C13 and C3, strains which produced acid normally in all milks).

II. PRESENCE OF ANTIBODIES IN INHIBITORY MILK

(a) Methods

Several methods were used for testing for the presence of antibodies in milk. These were agglutination, precipitation and ring tests.

(i) Agglutination Tests

Agglutination tests were carried out by the following two methods:

(1) Whey was prepared according to the method of Hall and Learmonth (1933). The clear whey so obtained was serially diluted with 0·85 per cent. saline. An equal volume of a standardized suspension of formalized cells (1,000 x 10^6 organisms per ml) was added to each dilution. As some of the organisms did not form stable suspensions in 0·85 per cent. saline, they were resuspended in decreasing concentrations of saline down to and including 0·15 per cent. All strains were shaken with glass beads in an endeavour to obtain stable suspensions. Tests were incubated (*a*) by overnight incubation at 55°C as used by Hobbs (1939), and (*b*) according to the method of Hucker (1932), who allowed antigen-serum mixtures to be incubated at 37°C for 4 hr. The tests were then removed from the water-bath, shaken, and held in a refrigerator overnight. Tests were then read for the degree of agglutination.

(2) As several workers have used the microscopic test as a means of judging whether agglutinins are present in the milk, a similar study of the growth of both susceptible and resistant strains in pasteurized wholemilk, pasteurized skim-milk and sterilized wholemilk was carried out. Smears were made from activity tests of susceptible and resistant strains which had been set up to include all three types of milk. Smears were made after 3 hr and 6 hr. These were stained with Newman's stain and examined.

(ii) Precipitation Tests

The Lancefeld precipitation test was used with Fuller's (1938) formamide modification. The antigen after extraction was carefully layered onto the surface of the whey, so that the line of demarcation between the two liquids was easily visible. A ring of precipitate at the junction of the two liquids was regarded as a positive test.

(iii) Ring Tests

The ring test, as used for the detection of Brucella agglutinins, was employed, and the method described by Wood (1948) was adapted for the purpose. This test utilizes a stained antigen. If the test is positive this antigen forms a highly coloured ring in the cream layer. A negative test is denoted by a uniform mauve colour throughout the milk.

(b) Results

Results were obtained over a range of milks, using a number of resistant and susceptible strains. All milks gave fair uniformity of results.

Agglutination tests were partially unsatisfactory because it was impossible to obtain stable suspensions of the susceptible strains. However, results with these strains after four hours indicated that no specific antibodies were present. Agglutination tests when the resistant strains were used gave completely negative results.

Microscopic examinations revealed several interesting features. Starters of both types which formed long chains in pasteurized milk (whole or skim) did so in sterilized milk. Both long-chain and short-chain susceptible strains were agglutinated in wholemilk and skim-milk, but the agglutination of short-chain susceptible strains was completely absent in sterilized milk. The long-chain susceptible organisms displayed a tendency to clump in both pasteurized and sterilized milk, this tendency being more pronounced in pasteurised milk.

In the case of resistant strains, however, short-chain organisms did not agglutinate in any of the milks, but strains forming long chains clumped in all milks, this clumping being more pronounced in the pasteurized than in the sterilized milk. It is also noteworthy that it was not possible to discern any difference between the microscopic results after three and six hours.

The results are summarized in Table 1.

Table 1

MICROSCOPIC APPEARANCE OF "SUSCEPTIBLE" AND "RESISTANT" STRAINS IN PASTEURIZED WHOLEMILK AND SKIM-MILK AND STERILIZED WHOLEMILK

Milk	Resistant Strain		Susceptible Strain	
	Long-chain Type C13	Short-chain Type C3	Long-chain Type HP	Short-chain Type R1
Pasteurized wholemilk ..	++	−	++	++
Skim-milk	++	−	++	++
Sterilized wholemilk ..	+	−	+	−

++ Pronounced clumping.
+ Slight clumping.
− No clumping.

As was to be expected in view of the results obtained with the agglutination tests, the precipitation tests using Lancefeld's extracts were found to be negative for all strains of organisms examined.

The ring test, on the other hand, was shown to be positive for all susceptible strains, but negative for the resistant ones. All tests were negative when carried out with sterilized milk.

The ring test, which was designed for the detection of brucellosis, is comparatively new and much work has been done with it over the past few years. Some of the data regarding it are conflicting. Diernhofer and Simetsberger (1953) found that although agreement between all the tests for detecting Brucella antibodies were good, the ring test was the least sensitive. On the other hand, Krejci (1956) found the ring test to be the most sensitive of all tests. Tallman and Herman (1954) in a comprehensive study of this test showed that production of a specific coloured antigen is the most critical step involved in the ring test procedure, and that the selection of a smooth *Brucella abortus* culture is of paramount importance. Antigen specificity was decreased when antigens were prepared from rough strains. It is of interest that Kastli (1955) found that Brucella cultures tested by him could be divided into two main groups differing in sensitivity.

Apparently Fleischhauer (1954) has been the only worker to adapt the ring test for the detection of serological infections caused by genera other than *Brucella*. He showed that this test did not yield consistent results in the examination of the sera of cows and horses for salmonellae infections. Keogh (1958) and McPhillips (1958) adapted the ring test in the same way as described herein, but gave no information as to the specificity of the antigens used by them.

In view of these results it seemed necessary that the starter cultures should be examined in order to determine whether they were "rough" or "smooth" in type. Acriflavine tests were therefore carried out on all organisms tested according to the method described by Stokes (1955). It is noteworthy that all susceptible strains tested were rough and all resistant strains were smooth.

Attempts were made to obtain smooth resistant variants from the rough susceptible strains by the use of (*a*) homologous antisera and (*b*) bacteriophage, but these were unsuccessful. In cases where smooth cultures were produced, the activity of these organisms in sterilized milk was low and therefore such cultures were useless for further testing for acidity production. Consequently, it was impossible to determine whether smooth-type cells obtained from the susceptible strains would behave like the resistant strains in pasteurized milk.

During the course of the work opportunity was taken to examine a number of the experimental bulk milks for the presence of agglutinins active against *Brucella abortus*. All such tests were positive, some presumably as a result of

Strain 19 vaccination. In view of this result it has been considered whether the positive ring test obtained with lactic streptococci was the result of cross-agglutination with *Brucella abortus* antibodies. In order to prove this point some preliminary testing was carried out with milk from experimental cows not giving positive agglutination tests. Milk from these cows gave a positive ring test (using a Streptococcus coloured antigen) and were found to be inhibitory to susceptible streptococcal strains. In the face of these results it can no longer be supposed that the positive ring tests were due to reaction with Brucella agglutinins.

III. EFFECT OF ADDING HOMOLOGOUS ANTISERA

As it is evident that the susceptible strains are carried up with the fat into the cream layer, presumably as a result of non-specific agglutination, it seemed desirable to determine the behaviour of a resistant culture in the presence of its specific antibody. Such an investigation was therefore carried out.

(a) Methods

Antisera were prepared in rabbits according to the method of Hucker (1932). The animals were inoculated on the 1st, 5th, 7th, 11th and 13th days. Increasing inocula of a standardized suspension of cells were used. At the first inoculation only 0·5 ml was given; the dose was increased by 0·5 ml at each inoculation. Eight days following the final inoculation, a test bleeding was made and the serum titre determined. Titre obtained was 1 in 1,250. Antisera were prepared against both susceptible and resistant strains. In the preparation of these antisera it was found that agglutination tests for the susceptible strain had to be read after four hours because these organisms did not form stable suspensions and auto-agglutinated on prolonged standing.

Activity tests were set up, using a resistant strain in pasteurized milk with homologous antiserum of determined titre, and sufficient to cause agglutination of this strain was employed.

Unfortunately, a limited supply of rabbits allowed the preparation of anti-serum against only one resistant and one susceptible strain.

Further information was needed to ascertain whether the resistant strain in the presence of its antibody was being taken up with the fat. Tests to determine this point were therefore set up in pasteurized milk, using separating funnels as culture bottles. Parallel tests were also set up with the susceptible strain and its homologous antibody. Smears were made of all tests at hourly intervals from three hours onwards. The smears were stained with Sudan IV and counterstained with Loeffler's methylene blue, then mounted in glycerine jelly. After six hours' incubation, the milks were taken out of the incubator and the skim-milk and cream fractions separated and retained. Titrations were then performed on all samples.

Photomicrographs were taken of the smears, using an "Ortholux" microscope and a "Praktica" camera.

(b) Results

Results of the preliminary tests with the resistant strain are set out in Table 2.

Table 2

PERCENTAGE ACIDITY PRODUCED BY A RESISTANT STRAIN (C3) IN PASTEURIZED MILK CONTAINING HOMOLOGOUS ANTISERUM

	Percentage Acidity Produced by Resistant Strain C3
Pasteurized milk	·70
Pasteurized milk containing homologous antibody ..	·28

The results show that the presence of its antibody caused a great drop in acidity production by the resistant strain. Further tests, involving separation of cream and skim-milk fractions, revealed quite definitely that agglutination of the resistant strain by its homologous antibody had taken place and had caused most of the cells to be taken up into the cream layer by the rising fat globules. Thus it caused the resistant strain to behave in all respects like a susceptible strain. These results are set out in Table 3.

Table 3

ACIDITY PRODUCTION OF SUSCEPTIBLE AND RESISTANT STRAINS IN MILK CONTAINING HOMOLOGOUS ANTIBODY

Milk	Percentage Acidity			
	Susceptible Strain		Resistant Strain	
	Skim-milk	Cream	Skim-milk	Cream
Control milk	·22	·40	·39	·32
Test milk (containing homologous antibody) ..	·22	·43	·26	·47
	Wholemilk		Wholemilk	
Control milk (mixed at end of test)	·22		·39	
Test milk (mixed at end of test)	·22		·26	

Susceptible Strain—HP
Resistant Strain—C3

It is quite evident also that the presence of homologous antibody had no effect on the acidity production of the susceptible strain in pasteurized milk. Results were the same, indicating that the concentration of the inhibitory properties native to milk were of such an order that additions of homologous antibody to the susceptible strain had no further effect on acidity production.

Microscopic examination of smears showed that both resistant and susceptible strains in the presence of their homologous antibodies were agglutinated in the skim-milk fractions following three hours' incubation. After six hours, the majority of organisms were in the cream layer and only a few remained in the

skim fraction. This result is identical with that already obtained when the susceptible strain was grown in pasteurized milk without antiserum. The resistant strain when grown in pasteurized milk without antibody followed the same pattern as has been described previously, i.e. the bulk of the organisms remain in the skim-milk fraction.

Photomicrographs of the resistant strain in the skim-milk fraction both with and without antibody serve as an interesting comparison with the tabulated results (Figures 1 and 2).

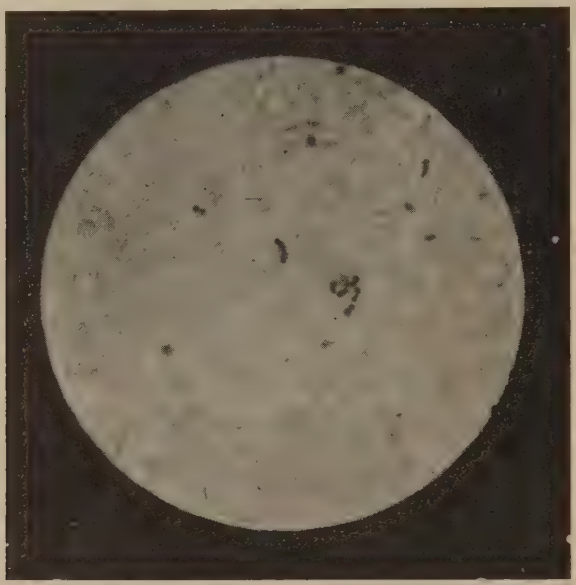

Fig. 1.—Resistant strain (C3) grown in pasteurized milk in presence of antibody for six hours at 30°C. Smear made of skim layer. Agglutination of the organisms has resulted in their rising with the fat. Sudan IV and methylene blue. X1140.

Some difficulty was experienced in obtaining smears uniformly stained by means of the Sudan IV method. This difficulty was aggravated by decolourization in preparing permanent mounts in glycerine jelly.

IV. EFFECT OF SPECIFIC AGGLUTININS

As no clear-cut evidence as to whether the presence of specific agglutinins in pasteurized milk was a possible cause of the susceptible strains rising with the fat was forthcoming from the trials, a series of tests was set up to investigate this problem.

Fig. 2.—Resistant strain (C3) grown in pasteurized milk without antibody. Smear made of skim layer. Without agglutination the organisms remain evenly distributed. Sudan IV and methylene blue. X1140.

(a) Methods

Milk was recombined, using 3 parts of sterilized skim-milk to 1 part of cream from pasteurized milk. It was found that this cream had to be heated at 76°C for 30 min in order to destroy any inhibitory properties attached to the fat globules, which were found to interfere with the tests. Activity tests were set up, using a susceptible strain, so that one test acted as a control while the other was inoculated with homologous antibody. A parallel series of tests was set up, using sterile skim-milk only. Microscopic checks were made for evidence of agglutination in the tests containing antibody.

(b) Results

Results of the tests are given in Table 4.

Acidity production by the susceptible strain in sterile skim-milk was unaffected by the presence of antibody, even though there was visible evidence of agglutination (shown by microscopic examination). However, the presence of antibody in recombined milk caused a decrease in acidity production; this was found to be due to the aggregation of the susceptible strains in the cream. This

Table 4

PERCENTAGE ACIDITY PRODUCED BY A SUSCEPTIBLE STRAIN (HP) IN RECONSTITUTED MILK CONTAINING HOMOLOGOUS ANTISERUM

Milk	Percentage Acidity Produced by Susceptible Strain
Sterile skim-milk	·45
Sterile skim-milk + antibody ..	·44
Recombined milk	·45
Recombined milk + antibody ..	·37

aggregation and the degree of inhibition, however, did not appear to be as marked as would be expected in pasteurized wholemilk. It is possible that this was due to the dilution of the antibody or alternatively the denaturation of the fat globule membrane by heat treatment, which may have interfered with the carrying-up of the organisms. This latter possibility is unlikely in view of the results previously obtained with heated cream and unheated skim-milk (Wright and Tramer 1957). It must be pointed out that the presence of skim-milk is a possible complicating factor.

V. DISCUSSION

As the susceptible strains were found to be rough in type while the resistant strains were smooth, any agglutination of the susceptible organisms would probably be non-specific.

In spite of the difficulties encountered in testing for the presence of agglutinins to the susceptible strains in the milk supplies, there is some evidence from both the direct microscopic test and the ring test which points to their presence. On the other hand, all tests used (agglutination, precipitation and ring tests) have consistently failed to show the presence of antibodies to any of the resistant strains in the milk supplies.

The agglutination test, although of limited use owing to the auto-agglutination of the susceptible strains, was still of value in estimating the antibody content of antiserum prepared against a susceptible strain. The results of agglutination tests (using various dilutions) differ from those obtained by Portmann and Auclair (1959), who obtained positive slide agglutination tests when several starter strains were used.

Because the agglutination tests were negative it would be expected that the precipitation tests would give similar results. This was found to be the case.

The use of the direct microscopic test for the study of the presence of agglutinins has, however, a very limited value, due to the fact that all long-chain organisms have a tendency to clump irrespective of the medium of growth. In

the case of the long-chain resistant strains, it has been proved that the increased clumping in pasteurized milks bears no relation to the presence of agglutinins. With the short-chain susceptible strains, it is likely that clumping in pasteurized milk is indicative of agglutination.

Although the specificity of the ring test is open to question in view of the fact that "rough" antigens were used, it is significant that negative results were obtained in sterilized milk even though the same antigens were employed. It is also of interest that McPhillips (1958) presented evidence demonstrating the specificity of the agglutination of "slow" starter cultures.

In this present work it was possible to bring about inhibition of the resistant strain by the addition of homologous antibody to raw or pasteurized wholemilk and this is proof in itself that specific antibodies are capable of bringing about an inhibition which is similar in every way to that normally occurring with the susceptible strains in pasteurized milk. These results were confirmed by microscopic evidence because the resistant strain plus homologous antiserum was agglutinated and carried up with the cream layer in the same way as a susceptible strain grown in pasteurized milk (without added antiserum). The similarity is even more marked when it is considered that the behaviour of a susceptible strain in the presence of homologous antibody shows the return of slight inhibition in milk reconstituted from fractions heat-treated to destroy inhibition.

It is also of interest that the addition of homologous antiserum to sterile skim-milk containing a susceptible strain caused marked visible agglutination but at the same time did not bring about any inhibition in acid production by the susceptible strain. Inhibition was only apparent when a cream layer was present. These results indicate that agglutination in itself is not responsible for inhibition and confirm conclusions reached by Wright and Tramer (1957) and Keogh (1958).

The agglutination in milk and whey of such a wide spectrum of bacteria as has been mentioned in the relevant literature has caused some speculation and has not been satisfactorily explained. Recently Portmann and Auclair (1959) found a constant relationship between the inhibition and agglutination of certain strains of *Streptococcus cremoris* and they suggested that both may be due to the same cause. Most workers, including Hobbs (1939), Rosenau and McCoy (1908) and Chambers (1920), have assumed that agglutination is non-specific. Indeed, it seems quite likely that many of the recorded instances of agglutination are merely the result of non-specific immunity occurring in the cow. Nevertheless, there is no doubt that the presence of agglutinins to such organisms as *Brucella abortus* and the pyogenic streptococci are the result of active infection. Although the results herein show that the positive ring test obtained with the lactic streptococci was not the result of cross-agglutination with *Brucella abortus* antibodies, the possibility exists that there may well be shared antigens between these strains of *Streptococcus cremoris* and with other Streptococci.

Although it is evident that the presence in milk of specific antbiodies to resistant and susceptible strains can result in their inhibition, there is no conclusive proof available at present that the inhibition of the susceptible strains as studied is due solely to antibodies (specific or non-specific).

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TECHNICAL NOTES

BANANA RUST THRIPS AND SCAB MOTH CONTROL TRIALS IN NORTH QUEENSLAND

The banana rust thrips, *Scirtothrips signipennis* Bagn., is a persistent pest of bananas in North Queensland, causing skin blemish which may seriously impair market value. The scab moth, *Nacoleia octasema* (Meyr.), is of less importance but may occasionally cause serious damage within a plantation: the larvae feed on the skin of the fruit and as with rust thrips market value is reduced.

The control of banana rust thrips has been given considerable attention, mostly in south Queensland, by several authors (Girault 1925; Veitch and Simmonds 1929; Smith 1933, 1934; Veitch 1934; Caldwell 1938, 1946; Smith 1947; Smith and Weddell 1949; and Saunders 1961). Results were adapted to northern conditions (unpublished records of the Department of Agriculture and Stock), and eventually a DDT/BHC dust was recommended for the control of both rust thrips and scab moth.

During March 1959 two trials were establised at East Palmerston, via Innisfail, North Queensland, to compare the efficacy of the standard dust with those of other materials which in recent years have been given some local publicity.

The materials used were:

Lindane.—An emulsifiable concentrate containing 16 per cent. w/v active ingredient.

DDT.—An emulsion concentrate containing 25 per cent. w/v *p.p'* isomer.

DDT/BHC.—A dust containing 2 per cent. *p.p'* isomer DDT, 2 per cent. BHC (0.26 per cent. gamma isomer) and an inert filler.

Dieldrin.—An emulsifiable concentrate containing 15 per cent. w/v active ingredient.

Methoxychlor.—An emulsifiable concentrate containing 22·5 per cent. w/v active ingredient.

"Telodrin" (WL1650).—An emulsifiable concentrate containing 15 per cent. w/v active ingredient (1, 3, 4, 5, 6, 7, 8, 8—octachloro—3a, 4, 7, 7a—tetrahydro —4, 7—methanophthalan).

Thiodan.—An emulsifiable concentrate containing 20 per cent. w/v active ingredient.

The field layouts were 10 x 4 randomized blocks with single-plant plots. On each trial site mature plants (see Figure 1) which had not thrown bunches were selected in four rows. As the bunches appeared in the throats of the plants the first treatment applications were made. Each treatment with the exception of Treatment 5 was applied weekly for the first three weeks, then at fortnightly intervals until harvesting. In Treatment 5 the entire pseudostem and the emerging bunch were sprayed once only.

Fig. 1.—Type of Cavendish plant used in trials at East Palmerston, 1959.

Spray applications were carried out to visible wetness with a Junior knapsack sprayer. The dust treatment was applied with a hand duster, and although care was taken to avoid heavy accumulations of dust on the fruit some did occur.

At harvesting (July-October 1959) the hands were removed from the bunches, and each finger rated for damage, the ratings for rust and scab moth being made separately. The following ratings, as percentage skin surface damaged, were used: 0, clean; 1, 1 to 25; 2, 26 to 50; 3, 51 to 75; 4, 76 to 100.

The percentages of fingers exhibiting either insecticide burn or spotting were recorded, and with other results are presented in Table 1.

Table 1

TRIAL RESULTS : INSECT DAMAGE AND PHYTOTOXICITY RATINGS

Treatment*	Trial 1			Trial 2		
	Average Rating		Fruit Burnt or Spotted (%)	Average Rating		Fruit Burnt or Spotted (%)
	Rust	Scab Moth		Rust	Scab Moth	
1. Standard, DDT–BHC dust	0	0	0	0·01	0	4·2
2. DDT spray 0·1 ..	0	0	0	0·04	0·01	0
3. Lindane spray 0·04 ..	0·03	0	0	0·03	0·01	7·5
4. Dieldrin spray 0·05 ..	0·01	0	0	0·02	0·08	30·3
5. Dieldrin spray 0·05 ..	1·02	0·10	4·8	1·60	0·35	0
6. Methoxychlor spray 0·06	0·05	0·41	10·7	0·10	0·05	39·9
7. Thiodan spray 0·06 ..	0·42	0·07	66·5	0·78	0	82·8
8. "Telodrin" spray 0·1	0·48	0	51·6	0·73	0·01	85·6
9. (10) Check	1·52	0·82	0	2·25	0·01	0

* All spray strengths as per cent. active ingredient.

These results and observations of field practices over some years are the basis of the recommendations by Saunders (1961) for the control of banana rust thrips and scab moth in North Queensland. A change from the standard DDT/BHC dust is not warranted, although as an alternative a DDT spray, 0·1 per cent., may be used.

Fig. 2.—Burning and spotting caused by insecticide spray.

The application of dusts and sprays is hampered by wet weather at the time of the year when the pests are likely to be prevalent (Saunders 1961). Accordingly care is required in the methods used, and the manner in which the materials are applied. Severe burning and spotting of fruit (Figure 2) may follow the use of certain insecticides (see Table 1, Treatments 4-8) or the injudicious use of those insecticides recommended. Dusting machinery should ensure an even flow at the outlet so that caking of large deposits on the fruit is avoided (note Trial 2, where 4·2 per cent. of fruit was blemished). DDT spray should be applied to the fruit only, as continuous treatment of the leaves may cause build-up of red spider mites (Tetranychidae).

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THE VALUE OF THE METHYLENE BLUE DYE REDUCTION TEST IN STUDYING NATURAL INHIBITORY PROPERTIES OF MILK

Although Leber (1950) introduced a resazurin test for starter activity, it was criticized by both Golding (1953) and Johns (1953) because milk which contained inhibitory substances such as penicillin gave normal dye reduction times even though titration results were low. Golding also stated that methylene blue was unsuitable for such work.

In spite of these criticisms it was considered that a modified methylene blue test with starter added might prove useful in the routine testing for naturally occurring inhibitory properties of milk, as such milk might well cause lengthened methylene blue reduction times by the starter. It was therefore decided to examine some of the starters which were inhibited in pasteurized milk (activity test) even though they gave normal acidities when used in the vitality test (Gillies 1957).

Both pasteurized and sterilized milks were dispensed in 10 ml quantities and 1 ml of a standard milk testing solution of methylene blue added to each tube. Both types of milk were inoculated with 1 ml of a 1/10 dilution of a 24 hr freshly clotted culture. Methylene blue tests were then carried out at 37°C. The tubes were examined every quarter-hour for reduction of the dye. Results were expressed in methylene blue reduction times. Activity tests were also set up in parallel according to the method of Anderson and Meanwell (1942).

As it was desired to carry out titrations every hour, in each case 2 ml of starter were added to 200 ml of milk. Acidity determinations were performed by first removing 10 ml samples aseptically and then titrating.

The starters used were all strains of *Streptococcus cremoris* and included strains which behaved normally in pasteurized milk as well as those which were inhibited.

Results of the tests are set out in Table 1.

Table 1

COMPARISON OF ACIDITY PRODUCTION AND DYE REDUCTION BY "SUSCEPTIBLE" AND "RESISTANT" STRAINS IN BOTH PASTEURIZED AND STERILIZED MILKS

Susceptible Strain—R6					Resistant Strain—C13				
Acidity Produced (%)			Methylene Blue Reduction Time (hr)		Acidity Produced (%)			Methylene Blue Reduction Time (hr)	
Hours	Pasteurized Milk	Sterilized Milk	Pasteurized Milk	Sterilized Milk	Hours	Pasteurized Milk	Sterilized Milk	Pasteurized Milk	Sterilized Milk
0	·16	·17			0	·16	·17		
3	·18	·20			3	·18	·19		
4	·20	·27	1¾	1½	4	·21	·24	1¾	1½
5	·22	·38			5	·32	·30		
6	·24	·50			6	·46	·40		

It is apparent from these results that there is no correlation whatever between acidity developed by any of the cultures in pasteurized or sterilized milks and the methylene blue reduction times. Susceptible and resistant strains gave the same dye reduction time in pasteurized milks, yet the acidity developed by the susceptible strain was approximately half that developed by the resistant strain.

However, when it is considered that reduction of the methylene blue occurred before any differences in acidity production were apparent, such a test is no real indication of any inhibitory properties in the milk. Accordingly, decreased inocula were used, so that the methylene blue times in both types of milk were increased and it was therefore possible to obtain a true comparison between the methylene blue reduction and the acidity developed. Such a series of results is expressed in Table 2.

Table 2

COMPARISON OF METHYLENE BLUE REDUCTION TIMES OF "SUSCEPTIBLE" AND "RESISTANT" STRAINS IN PASTEURIZED AND STERILIZED MILK USING DIFFERENT INOCULA. (ACTIVITY TEST RESULTS ARE ALSO INCLUDED)

	Methylene Blue Reduction Times in Hours			
	Susceptible Strains		Resistant Strains	
	HP	R6	C2	C3
Sterilized milk—				
1% inoculum	¾	¾	1	1½
·2% inoculum	1¾	2¼	1½	1½
Pasteurized milk—				
1% inoculum	4½	6	2¼	2½
·2% inoculum	>7½	>7½	4	4
	Percentage acidity developed using 1% inoculum			
Sterilized milk	·38	·43	·42	·46
Pasteurized milk	·22	·22	·36	·40

It is evident from Table 2 that it is possible, by the use of decreased inocula, to obtain a methylene blue reduction time which correlates with the acidity developed by the susceptible strains in pasteurized milks. It is also apparent that the reduction times for all strains (susceptible and resistant) are longer in pasteurized milks than in sterilized milks, evidently due to changes in the reducing systems by sterilization. In this point the two tests differ, as resistant strains are able to produce comparable quantities of acid in both pasteurized and sterilized milks.

Although it has been possible to obtain methylene blue reduction times which reflect the presence of naturally occurring inhibitory properties in the milk, the time taken to complete both activity and methylene blue reduction tests is comparable. The principal advantage in the methylene blue reduction test lies in the fact that it is more easily adapted to field use than the activity test.

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